MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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HISTOPLASMA DUBOISII AND AFRICAN HISTOPLASMOSIS

R. VANBREUSEGHEM [1]

(WITH 6 FIGURES)

In a recent paper, A. Dubois, P. G. Janssens and P. Brutsaert (**1952**) have described the first case of histoplasmosis in the Belgian Congo. The mycological description of the parasite was made by myself (**1952**) and I considered on the basis of the parasitic as well as of the saprophytic characters that I could make a new species of this fungus, *Histoplasma duboisii*, previously undescribed as new. As a matter of fact, a careful study of the literature, as well as personal correspondence with my friend J. T. Duncan, has revealed that the same parasite has probably been encountered several times and always in cases of African origin. Lecéne (cited by Brumpt, **1936**), A Catanei and P. Kervran (**1945**), P. Kervran and R. Aretas (**1947**), Friess and Delvoye (**1947**), J. T. Duncan (**1947**), and, finally Dubois, Janssens and Brutsaert (**1952**), have been, as far as I can judge, the sole authors who have described human cases of histoplasmosis in which the parasitic cells were very large (*duboisii* form) as contrasted with the small parasitic cells commonly encountered in classical histoplasmosis (*capsulatum* form). In the second edition of the

[1] This investigation was made possible by financial assistance from the Institut pour la Recherche Scientifique en Afrique Centrale.

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Précis de Mycologie (Langeron and Vanbreuseghem). I have already given a brief discussion of histoplasmosis of African origin, and it seems that there is some reason to speak of an African histoplasmosis caused by *Histoplasma duboisii* as distinct from the classical histoplasmosis caused by *Histoplasma capsulatum*. This problem has been recently discussed by A. Dubois and R. Vanbreuseghem (**1952**).

It is probably not out of place to give some additional and more complete information on this interesting parasite.

The patient who was the source of the new strain was a white male of Belgian origin who had been for the last six years engaged in agricultural work in the central part of Belgian Congo. Some six months before his return to Belgium, swelling of the lymph nodes had been noticed in the neck and he had suspected that they were the first symptoms of African sleeping sickness. Later on, swelling of lymph nodes developed in the axilla. Puncture of the lymph nodes and histological examination of a node taken by biopsy in the cervical region revealed the presence of large and numerous parasitic cells which, afterwards, were also found in the sputum. These cells were for the most part included in large histiocytes or free in the surrounding medium. A search for the cells in the sternal marrow remained entirely negative.

MORPHOLOGICAL FEATURES OF THE PARASITIC CELLS

The parasitic cells as they were encountered in the lymph nodes of the patient are ovoid cells 13.5 $\mu \times 10\,\mu$. They possess a thick double-contoured capsule, 1–2 μ, and contain a very large amount of fat in the form of a unique, perfectly round globule which fills the cell almost completely, or of smaller globules, sometimes very numerous, of a spore-like appearance. Their real nature is, nevertheless, easy to demonstrate by mixing one drop of pus with one drop of Cotton Blue-Sudan in Chlorallactophenol, which gives to the fat a mahogany color, or by covering a dried smear of the pus with a 2 percent solution of osmic acid, which colors the fat grey. The Cotton Blue-Sudan—which, like the osmic acid, must act a long time (20 to 24 hours) to reveal the fatty nature of the inclusions—demonstrates at the same time some protoplasm in the

cell. When, exceptionally, the cell is devoid of fat, this appears as fine blue granulations dispersed in the cell or, when the cell contains a larger fatty globule, orients itself at its two poles.

The thick capsule is most often quite smooth; sometimes it is thicker than usual and, in such cases, it seems to be made of stratified layers, like a starch grain. Under careful observation these layers may show some cleavage and in these particularly

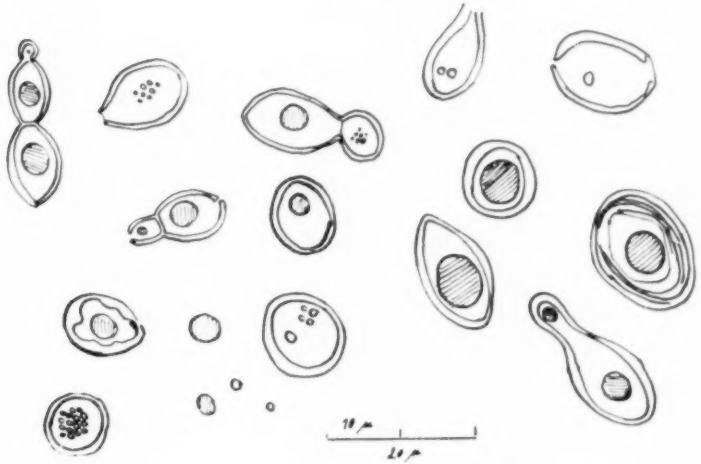


FIG. 1. *Duboisii* forms of *Histoplasma duboisii* from the lymph nodes of the patient, July, 1952.

thick capsules one may notice occasionally a notch in their most superficial layers.

Beside these typical cells one finds rather numerous budding cells. The bud begins on the smallest of the two poles of the ovoid cell. A thin membrane encloses it at the very beginning; this membrane seems to be the internal layer of the capsule of the mother cell and sometimes chains made of 4 or 5 successive cells may be found.

Gradually, in proportion as it grows, the membrane of the daughter cell becomes thicker and transforms itself into a capsule identical with the one of the mother cell; it follows that one may find in the smear, chains of 3 to 5 cells of the same size and of the

same morphological features. It is then impossible to decide which of the two terminal cells has been the source of the others.

In the sputum, the parasitic cells were rare and their size smaller than those of the lymphnodes (about 10 μ in their greatest diameter). The fat in these cells was generally more dispersed than in the cells of nodal origin.

In none of the examinations made of the pus from the lymph nodes, or of the sputum, was the classical small parasitic form of *Histoplasma capsulatum* encountered.

CULTURES OF HISTOPLASMA DUBOISII

It was quite easy to cultivate the fungus from the pus of the lymph nodes. On Sabouraud agar (glucose 2 percent) or on blood agar (sheep or rabbit blood), I secured a fair development at 25° C, maybe a little faster on the blood agar than on the Sabouraud agar. It takes, nevertheless, about 10 to 15 days to have a well developed colony. Although the richness of the pus in parasitic cells was enormous, the number of colonies obtained per slant did not exceed a good tenth. From the first cultures it was easy to obtain subcultures on the same medium or on glucose broth, soil agar with or without sugar, and even on Smith's synthetic medium.

MACROSCOPIC MORPHOLOGY OF THE COLONIES

1. *On Sabouraud agar (glucose 2 percent).*

At their very beginning the colonies look just like ground glass. In a few days they become white and downy, and grow very regularly, producing above the slant surface a disk 2–3 mm in thickness. The subcultures do not assume the ground glass appearance and are, at first, white and downy. With aging, the colonies become powdery and of a brownish or ochraceous color: this particular appearance is the result of the development of very numerous chlamydospores. Examined from the bottom, through the agar, the colonies, lightly immersed in the medium, show a chestnut color.

The centrum of the colonies is sometimes gently umbilicate, sometimes elevated above the rest of the colony.

There is a tendency to pleomorphic degeneration, variable from one culture to another, and it is necessary to make subcultures every 3 or 4 weeks to maintain the strain in normal condition.

2. On blood agar.

On blood agar, the colonies remain very superficial and develop like a thin membrane; later, they become brown and powdery but they never acquire the thickness they have on Sabouraud agar.

3. On soil medium.

On Sabouraud agar enriched with soil and on "conservation" medium enriched with soil, the growth of *Histoplasma duboisii* is very much the same as on plain Sabouraud. On soil agar—i.e., on a medium containing as sole nutrients: soil, agar and water—the development of the fungus is scanty but evident.

4. *In liquid medium.*

The growth starts in the deep layers of the liquid; the colonies are composed of downy mycelium, spherical or, when they adhere to the wall hemispherical. At a later stage, the superficial layer of the medium is invaded by the fungus; the colonies look like wax and are honeycombed, but at the margin they are powdery. In Smith's medium they are white, swollen and powdery.

MICROSCOPIC MORPHOLOGY OF HISTOPLASM DUBOISII

Most of our observations have been made on slide cultures made following the technic of Rivalier and Seydel. The observation is easy and the development of spores is abundant, perhaps more abundant than in slants. Every culture was grown at 25° C on Sabouraud agar. The inoculum was either the pus obtained from the patient or cultures obtained from this pus.

1. *Colonies developed directly from the pus.*

Most of the time, the result of inoculation on slides was a complete failure. In some cases we get a very slight growth of hyphae

but we have not been able to observe the development of those hyphae from the parasitic cells. These had completely disappeared after a week's incubation: sterile and most often coremioid hyphae had taken their place. It seems impossible to conclude from those observations anything other than the difficulty of obtaining primocultures on slides.

2. Subcultures on slides.

We started either with first cultures, 30 days old, or with subcultures 15 days old: the morphology of the colonies was the same in either case. The observation of slide cultures of different ages made it possible to obtain information on the structure of the colonies and among their constituents I have been able to recognize the following elements:

a. *The vegetative and aerial mycelium.*

The vegetative hyphae, about 2.5 μ in breadth, septate, with colorless walls, grow towards the periphery; they are slightly wavy. The length between two septa is 30 μ. Secondary vegetative hyphae arise from the principal hypha generally just behind a septum, but sometimes at a point as far as half-way between two septa. The angle made by the principal and the secondary hypha is wide and may be equal to a right angle. At the point where the secondary hyphae arise on the principal one, the latter quite often changes its direction of growth so as to correct the general direction of growth with its former line of growth. This aspect creates a false figure of dichotomy. Aerial hyphae arise on the principal hypha at a right angle; at their starting point, they make a cork screw-like movement. The space separating two septa is shorter in aerial hyphae than in vegetative hyphae; when they produce microconidia, the segments are no more than a few microns in length but when they produce macroconidia they are much larger (from 15 to 30 μ). The origin of the aerial hyphae is behind the septum just as in the case of the secondary vegetative hyphae; when the last ones have sufficiently grown they also may produce aerial hyphae.

b. *The spores: Microconidia and Macroconidia.*

Microconidia grow on both sides of the hyphae—generally aerial hyphae. They are pedunculate, round and their diameter is about 2.5 μ. The hyphae from which they arise are divided into short sections and the microconidia are borne, close to one another, or on opposite sides in rows of 20 to 30. When they are very numerous, the hyphae from which they arise is empty and is hardly visible; its figure is suggested by the position of the microconidia. The peduncle is very narrow (maximum, 1 μ) and no more than 2 μ in length. Exceptionally, one may see a microconidium arising from another one and I have found a chain of 3 microconidia attached one to the other.

Macroconidia are of two types: they are smooth, or covered with very irregular spines (echinulated chlamydospores). When ripe, their diameter is about 13 μ; they are prefectly round, and a slender sporophore, 1 to 2 μ in diameter, short, or up to 30 μ or more in length, connects them with the mother hyphae. The smooth macroconidia have a thick double-contoured membrane. The external wall is smooth and regular. The internal wall is smooth and regular but sometimes depressed on the contents of the macroconidia. The contents, sometimes clearly visible, are mainly composed of drops of fat. The chlamydospores or echinulated macroconidia have also a thick double-contoured membrane, but whereas the smooth and regular internal wall is scarcely visible, the outer one is covered with very irregular spinous overgrowths (2 μ long and 2 μ in diameter).

Are the microconidia related to the macroconidia, and the smooth macroconidia to the echinulated macroconidia? It is difficult to answer this question. I do not think that the microconidia are just small, unripened macroconidia: their position on the hyphae is very different. The sporophore of the microconidia is always very short which, on the contrary, is not the rule for the macroconidia. I do not think, either, that the smooth macroconidia are a mere stage in the formation of echinulate macroconidia. Actually, one can see in the cultures small echinulate macroconidia as well as large and mature ones. Besides, in old cultures, smooth macroconidia may be much more numerous than

echinulated chlamydospores. Furthermore, in my slide cultures, I have always seen the echinulated macroconidia before I could find any smooth macroconidia.

EXPERIMENTAL INOCULATION OF THE HUMAN PUS IN ANIMALS

White mice have been inoculated with pus diluted in physiological water (1 vol. pus/1 vol. water) intracerebrally (0.05 ml) and in the peritoneal cavity (0.25 ml). No symptoms developed. Guinea pigs have been inoculated with the pus (same dilution) in the peritoneal cavity (0.5 ml) or in the testis (0.2 ml). None of the animals became infected.[2]

It seems that it is impossible to transmit *Histoplasma duboisii* to the animal starting from the parasitic human cells. On the contrary, as may easily be ascertained, the inoculation of the saprophytic form is easy, at least to the guinea pig, when one makes use of the testicular route.

EXPERIMENTAL INOCULATION OF THE CULTURES OF HISTOPLASMA DUBOISII

The inoculum was made from 15 day old subcultures whose surface was gently rubbed with a glass rod in 3 ml physiological water per slant. The emulsion was composed mainly of spores.

Inoculation of white mice.

Six white mice were injected, in the peritoneal cavity, with 0.5 ml of the infecting emulsion. Two of them were killed 8 days later but nothing special could be noticed. Two others were killed 15 days after the inoculation. In one of the two, on the mesentery, small whitish nodules 1–2 mm in diameter were found. They contained very numerous parasites of the *capsulatum* form. The two last animals died but the autopsy could not be performed.

From this experience we may conclude that it is possible to inoculate *H. duboisii* in white mice but as far as we know the

[2] After this paper has been submitted, this inoculation was found possible. See R. Vanbreusegem, A. Dubois, P. Brutsaert and P. G. Janssens. Transmissibilité au cobaye d'Histoplasma duboisii à partir de la forme humaine. Proc. Soc. Belg. Méd. Trop. **33**: 171–176. 1953.

evolution of the parasite does not go farther than the *capsulatum* form stage. It is evident that other experiments must be undertaken before it is concluded that the *duboisii* form does not appear in the white mice.

Inoculation of rabbits.

Two rabbits received by the subcutaneous way 3 ml of the same emulsion used for the white mice. The next day a slight swelling was noticeable at the point of injection but a week later no more symptoms were observed. Nearly 7 months later one of the two

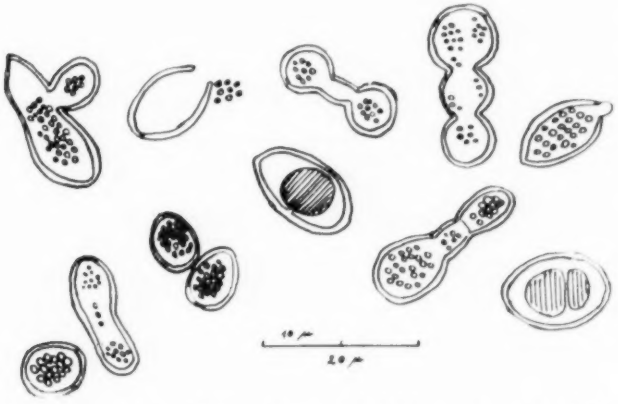
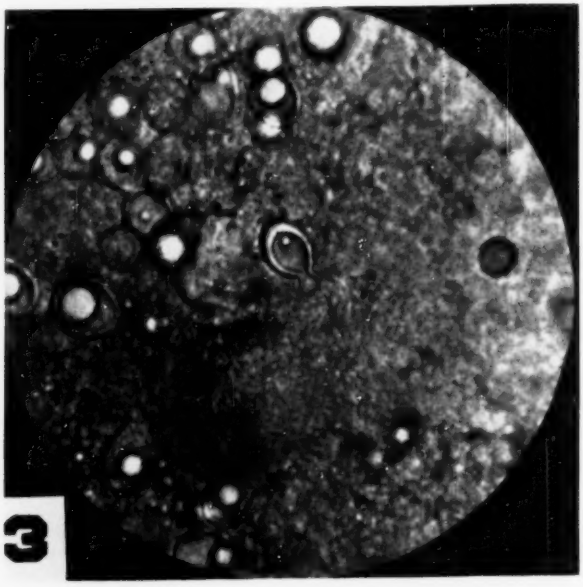


FIG. 2. *Duboisii* forms of *Histoplasma duboisii* from the testis of a guinea pig four months after inoculation.

animals died. An autopsy was performed. A small quantity of a clear effusion was found in the pericardic cavity and a yellowish tumor in one of the two lungs. Culture of this tumor did not give any result. The second animal was still alive and apparently healthy 8 months after the inoculation.

Inoculation of guinea pigs.

I made use of the testicular route and injected in the right testis 0.5 ml of the same emulsion utilized for mice and rabbits. Two guinea pigs were inoculated and about every week the testis was punctured with a fine needle. Eight days after inoculation, the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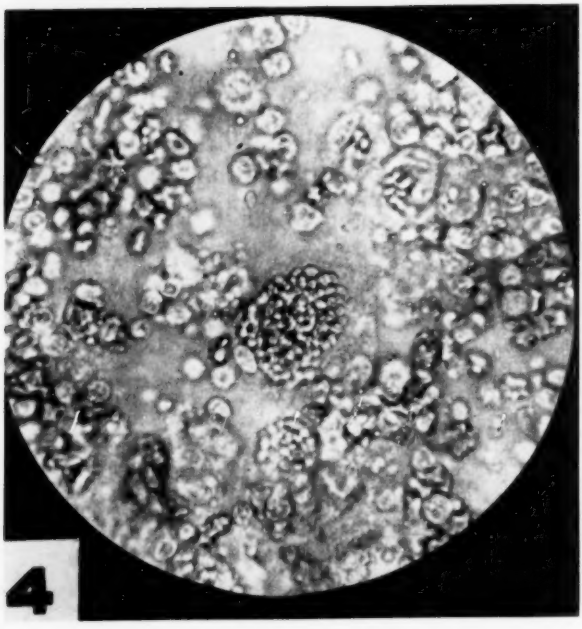
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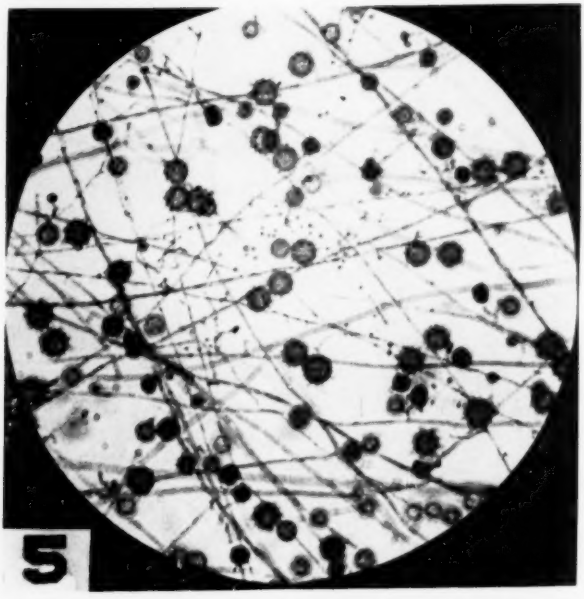
puncture was ineffective in one of the animals but in the other one showed some echinulated chlamydospores. Eight days later, i.e., 15 days after inoculation, numerous parasitic cells of the *capsulatum* form were found in the testis of both animals. Three weeks after inoculation the parasitic cells were always very numerous but a good many were of larger size than the typical parasitic cells of *Histoplasma capsulatum* and recall the morphological figure of the parasitic cells of *H. duboisii* as found in the patient.

Three weeks after the inoculation, cultures were obtained from the product of the puncture of the testis of both animals. Contrary to what happened with the human pus, the cultures from the testis of the guinea pigs were very abundant. It is to be noted that at this stage the *capsulatum* forms were still much more abundant than the *duboisii* forms.

About two months after the inoculation one of the two guinea pigs died. Necropsy revealed a slight hemorrhagic peritoneal exudate without any parasites. Parasites were found only in the inoculated testis, mainly in the *duboisii* form, much more rarely in the *capsulatum* form. The cause of death was not evident.

Four months after inoculation the second guinea pig was found to be severely ill. The eyes were injected; ulcers 2 mm × 4 mm were developed on the right inferior palpebra. Small yellowish tumors were discovered on both sides of the palpebral conjunctiva; numerous *duboisii* forms were found in the conjunctival sacs. In the neck as well as in the inguinal regions big lymph nodes had developed. The two testes had been replaced by large irregular masses as big as a half nut. An autopsy was performed (chloroform). The two testes had been pushed back in the abdominal cavity; they were enlarged as well as the epididymis. The scrotal masses found on the living animal were not the testes but lymph nodes. A large lymph node had developed on the back side of the right eye. Peritoneum, liver, lungs, spleen, right kidney seemed normal. The left kidney was enlarged. *Duboisii* forms

FIGS. 3, 4. 3. *Duboisii* forms in the lymph node of the patient. Stained with Cotton Blue-lactophenol, × 880. 4. *Capsulatum* forms packed in a large histocyte in the testis of a guinea pig 15 days after inoculation with *Histoplasma duboisii*, × 500.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were found in the two testes, liver, heart, left kidney, and in the lymph nodes. Cultures were easily obtained from the two testes. The parasites in the two testes, the lymph nodes and the left kidney were very numerous; they were of the same size as in the patient but their forms were more irregular.

Taxonomy of Histoplasma duboisii

It is rather evident that the fungus isolated from the patient of Dubois, Janssens, and Brutsaert possesses a good many features of its own when comparison is made with *Histoplasma capsulatum*, the agent of classical histoplasmosis. First of all, very large forms have been encountered in man and in guinea pigs which have never been encountered in man or animal parasitised with *Histoplasma capsulatum:* to this morphological aspect of the new parasite we gave the name of *duboisii* form. In the guinea pigs, a parasitic form which seems identical with the parasitic form of *Histoplasma capsulatum* precedes the appearance of the *duboisii* form; we gave to this morphological aspect of *Histoplasma duboisii* the name of *capsulatum* form. There is every reason to believe that the *duboisii* form evolves from the *capsulatum* form. As a matter of fact *capsulatum* forms which are the first to appear in the testes of the guinea pigs inoculated with *Histoplasma duboisii* enlarge progressively, their capsule becomes thicker and thicker and finally they attain the typical aspect of the *duboisii* form. The cultures of *Histoplasma duboisii* possess also characteristics of their own, the main one being the perfectly round form of the chlamydospores; piriform chlamydospores never being produced as is the case with *Histoplasma capsulatum*.

I am not yet able to decide whether *Histoplasma duboisii* is less pathogenic than *Histoplasma capsulatum*. Both parasites do not seem to possess a very large pathogenic power for animals. This aspect may be modified when we are able to obtain the yeast phase of *Histoplasma duboisii*, which has not yet been possible.

FIGS. 5, 6. 5. *Histoplasma duboisii*. Culture 20 days old showing numerous macroconidia and microconidia, ×285. 6. *Histoplasma duboisii*. Culture 20 days old showing four echinulate chlamydospores and numerous microconidia, ×880.

For the different reasons explained about, I thought it justifiable to create the new species *Histoplasma duboisii* Vanbreuseghem 1952. It seems from the citations made in the beginning of this paper that this species had been discovered by other workers but it had been generally considered to be *Histoplasma capsulatum*. Duncan was the sole author to suggest that it might be a new species. We will learn in the future if I was right or not and if there is sufficient reason to admit that there exists in Africa, besides the classical histoplasmosis of which *H. capsulatum* is the cause, another histoplasmosis, African histoplasmosis, produced by *Histoplasma duboisii*.

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FURTHER STUDIES ON THE FRUITING OF PHYSARUM POLYCEPHALUM

WILLIAM D. GRAY[1, 2]

(WITH 2 FIGURES)

In earlier papers (Gray, **1938**, **1939**) the writer demonstrated that plasmodia of the Myxomycete, *Physarum polycephalum* Schw., usually did not form fruiting bodies in the absence of visible light. From preliminary experiments conducted with radiations which were predominantly in narrow ranges of the visible spectrum it appeared that the shorter wave lengths of visible light were more effective in the initiation of sporangia formation than were the longer wave lengths. The work herein reported is an extension of the earlier work, employing light of known intensity from narrower regions of the visible spectrum.

EXPERIMENTAL PROCEDURE AND RESULTS

In these studies stock cultures were maintained by the moist chamber method described by Camp (**1936**), but the actual test cultures were starved cultures in beakers prepared as described in a previous report (Gray, **1939**). All experiments were conducted at 25° ± 1° C, and the initial pH value of the liquid in the beakers (McIlvaine's buffer, diluted 1 : 10) was 3.0.

As a source of light, a large mercury vapor lamp (with glass tube) was used, the radiations of various wave length being isolated by the insertion of a series of liquid filters between the lamp and a black paper lined chamber in which cultures were irradiated. The filter chamber series was built as follows: Four brass separators were made by bending square brass tubing (about ½ inch

[1] Department of Botany, The Ohio State University, Paper No. 552.

[2] The experimental work here reported was done during the writer's tenure as a National Research Council Fellow at the University of Wisconsin under the sponsorship of Prof. B. M. Duggar.

thick) into 19 × 19 inch squares, the open corners of which were brazed. Two ¼ inch holes were drilled through the tubing on one side and 3 inch lengths of ¼ inch (O.D.) brass tubing were soldered into each hole. The four brass separators were then laid alternating with five 20 × 20 inch squares of clear window glass, and wood frames were built to hold the glass panes, separated by brass squares, in position. The glass panes protruded about ½ inch beyond the brass separators all the way around, and the spaces between the protruding panes of glass were filled with a lanolin–resin glue, the preparation of which is described in a paper by Boeshore and Gray (**1936**). Thus, four watertight chambers (18 inches square and about ½ inch deep) resulted. Each of the chambers could be filled or drained independently of the others by means of the two round brass tubes which passed through the brass separator which formed the sides of that particular chamber.

Three wave bands of the mercury arc were employed: the blue line at 4360 Å, the green line at 5460 Å, and the two yellow lines at *ca.* 5780 Å. Liquids used in the filter chambers for the isolation of the blue and yellow lines were those recommended by Warburg (**1928**). The combinations of liquids used for isolation of the various wave bands were as follows:

ISOLATION OF THE BLUE LINE AT 4360 Å:

Chamber 1: 2% quinine hydrochloride
Chamber 2: 0.003% acid rhodamine
Chamber 3: Ammoniacal $CuSO_4$ prepared by adding normal NH_4OH to M/10 $CuSO_4$ until precipitation was complete
Chamber 4: 6% $CuSO_4$

ISOLATION OF THE GREEN LINE AT 5460 Å:

Chamber 1: 6% $CuSO_4$
Chamber 2: 0.22% $K_2Cr_2O_7$
Chamber 3: Neodymium ammonium nitrate solution
Chamber 4: Neodymium ammonium nitrate solution

The neodymium ammonium nitrate solution used in Chambers 3 and 4 in the isolation of the green line was not a solution of the

pure salt. The solution was prepared by heating 50 gms of a rare earth mixture (containing about 22 percent neodymium) in nitric acid on a steam bath until most of the liquid was evaporated; ammonium hydroxide was then added and most of the liquid was again evaporated. The process of heating first with nitric acid and then ammonium hydroxide was repeated several times until the soluble neodymium salt was formed. The soluble salt from 50 gms of rare earth mixture was dissolved in 325 ml of distilled water. When the absorption spectrum of this solution was examined, it was found that a 2 cm layer absorbed the yellow lines of the mercury arc but readily transmitted the green line. In addition to neodymium the rare earth mixture contained samarium, europium, and praseodymium; soluble salts of the latter probably aided in isolation of the green line, since praseodymium salts also transmit green light but absorb yellow light.

ISOLATION OF THE YELLOW LINES AT 5770 AND 5790 Å:

Chamber 1: 2% quinine hydrochloride
Chamber 2: 0.2% tartrazin plus 0.02% erythrosin
Chamber 3: 6% $CuSO_4$

Light intensities were measured by means of a thermopile–galvanometer system which had been calibrated against a standard lamp.

EFFECT OF WAVE LENGTH OF LIGHT UPON FRUITING. The effects of light of the above wave lengths upon percentage of fruiting and length of time necessary for fruiting (measured from the time at which the culture was placed under the light to the time that sporangia were mature) were ascertained by continuously irradiating beaker cultures of plasmodia with light passed through one of the liquid filter series. Results of these studies are presented in TABLE I.

From the results presented in TABLE I it is evident that sporangia formed in a greater number of cultures and the time necessary for the initiation and completion of the fruiting process was less when plasmodia were exposed to the shorter wave lengths of the visible spectrum. The possibility exists that, since the intensity of the radiation at 5460 Å was somewhat less than that at

TABLE I

THE RELATION OF FRUITING OF PHYSARUM POLYCEPHALUM TO WAVE LENGTH OF LIGHT

Wave length	Intensity (erg/cm²/sec)	Number of cultures	Number that fruited	Percentage fruiting	Average no. of hrs. necessary for fruiting
4360 Å	288	61	55	90.1	51.4
5460 Å	224	17	14	82.3	76.0
5770–5790 Å	392	38	9	23.7	120.0

4360 Å, the difference in time necessary for fruiting is not significant. However, the intensity of the radiation at 5460 Å was only about 22 percent less than at 4360 Å, whereas the length of the time period necessary for fruiting was about 48 percent greater in the cultures irradiated with the light of longer wave length. In an earlier paper (Gray, **1938**; see Tables I and 4) it was shown that a 50 percent reduction in light intensity resulted in only a 7 percent increase in time necessary for fruiting. Thus, the differences reported for the cultures irradiated with blue light and green light cannot be due entirely to slight differences in light intensity. The intensity of the yellow light employed was 75 percent greater than that of the green light, yet cultures irradiated with yellow light required a 58 percent longer time period for fruiting than those irradiated with green light. From this it would appear that wave lengths of the yellow region of the visible spectrum are considerably less effective than those of the green region.

Since visible light has such a marked effect upon this organism, it is obvious that the various light rays must be absorbed to a greater or lesser extent. In order to gain a possible explanation of the dissimilar effects of the different wave lengths, extracts containing the plasmodial pigment were prepared, and percentage absorption of various wave lengths of light was ascertained with a Bausch and Lomb Spectrophotometer. No attempt was made to obtain the pigment in pure condition, the solution being prepared by macerating 5 gms of fresh plasmodium in 25 ml of cold acetone and filtering the extract; thus the solution contained all of the acetone-soluble materials of the plasmodium.

Percentage absorption of different wave lengths of visible light by a 5 mm layer of this extract is presented graphically in FIG. 1. From the absorption curve in this figure it is apparent that the greatest percentage absorption occurs in the blue end of the visible spectrum. In view of the fact that the plasmodial pigment has an absorption spectrum of this nature a possible simple explanation of the results presented in TABLE I might be that a longer period of time is necessary for initiation and completion of sporangia-formation in the longer wave lengths because much smaller percentages of these wave lengths are absorbed. On the other hand, when percentages of yellow and green light absorbed are compared, there is further indication that qualitatively yellow light is less effective in this process than green light.

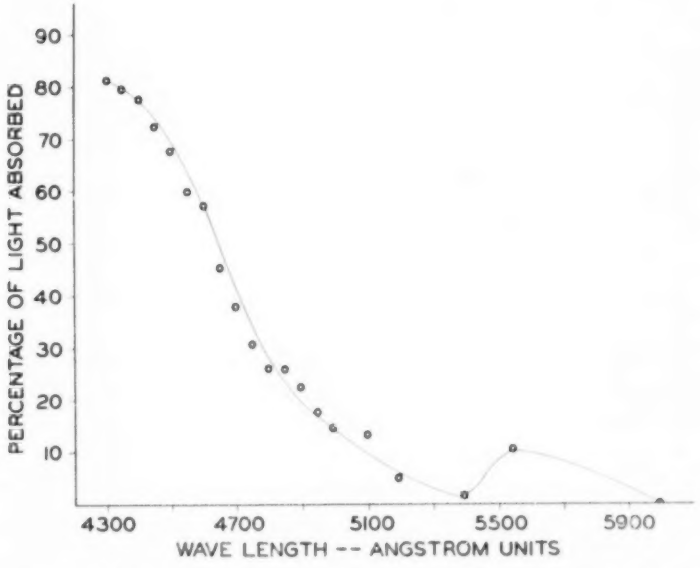

FIG. 1. The absorption of visible light of different wave lengths by an acetone solution of the pigment of *Physarum polycephalum*.

THE INTERRELATIONSHIP BETWEEN LIGHT ABSORPTION, pH AND FRUITING. In an earlier paper (Gray, **1939**) it was pointed out that in the pH range of 3.0–8.0, percentage of fruiting varies in-

versely with pH value, temperature and light intensity being constant. It was also reported that in the pH range 4.0–8.0, the length of time necessary for fruiting varies directly with pH value; i.e., the lower the pH value the sooner sporangia formed. Since the present work has shown that a relationship exists between fruiting and wave length of light, it was thought that perhaps the pH and light effects were in some way interrelated.

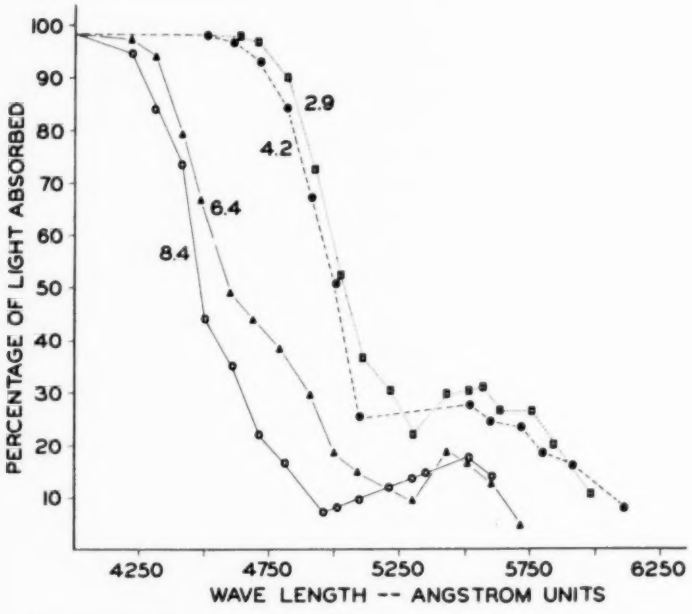

FIG. 2. The relation of pH to light absorption by the pigment of *Physarum polycephalum*.

Seifriz and Zetzmann (1935) have shown that the pigment of *Physarum polycephalum* is a natural pH indicator which changes color with pH changes in the surrounding medium. Since this is the case, it is reasonable to infer that changes in color which occur as a consequence of changes in acidity might also be accompanied by changes in light absorption. This point was investigated by preparing extracts by macerating 5 gms of fresh plasmodium in 25 ml of cold absolute methyl alcohol and filtering.

Five ml portions of such extracts were added to equal volumes of thallate buffer of various pH values, and the pH values of the resultant solutions were determined. The absorption curves which were obtained with 5 mm layers of four such solutions are presented in FIG. 2. From the curves presented in this figure it is evident that decreasing the acidity of the medium containing the pigment results in a reduction in light absorption by the pigment; this is especially noticeable in the violet and blue end of the visible spectrum. Such results provide a basis for the suggestion that the previously demonstrated pH effects may be due in part to the fact that increased acidity of the surrounding medium results in a change in the pigment in such manner that a greater quantity of light is absorbed by the organism. Such a course of events would inevitably lead to a more rapid initiation and completion of the fruiting process, since it has already been demonstrated that when other conditions are constant, time necessary for fruiting varies inversely with light intensity.

SUMMARY AND CONCLUSIONS

Plasmodial cultures of *Physarum polycephalum* Schw. were irradiated continuously with monochromatic light in the blue and green portions of the visible spectrum and a very narrow band in the yellow portion. It was found that the shorter the wave length of light the more rapidly the plasmodia formed fruiting bodies and the greater the percentage of cultures forming fruiting bodies.

When the absorption spectrum of a crude extract of plasmodium containing the pigment and other acetone soluble materials was determined, it was found that the greatest percentage of light absorption was in the blue end of the visible spectrum. Thus, the more rapid fruiting which was observed when plasmodia were irradiated with blue light of 4360 Å wave length was probably due to the fact that a much greater amount of light was absorbed, although there was some indication that longer wave lengths of light are less efficient in the initiation of the fruiting process.

Plasmodial extracts of different pH values had quite different absorption spectra: the greater the acidity of the extract, the greater the quantity of light absorbed; this was especially marked

in the range of 4000–5000 A. It is suggested that the accelerating effect which increased acidity exerts on the fruiting process may be due in part to the increased capacity for light absorption by the plasmodium when placed in medium of low pH value.

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THE UTILIZATION OF NUCLEIC ACID DERIVATIVES BY NEUROSPORA

FREDERICK T. WOLF

(WITH 1 FIGURE)

The utilization of amino acids by microorganisms has received a great deal of attention during recent years. With respect to the nucleic acid derivatives, another group of naturally occurring nitrogenous substances, microbiological studies have centered largely upon the role of these compounds as accessory growth factors. Consequently, relatively little is known about the microbial disintegration of the nucleic acids, and the role of their degradation products as sources of nitrogen for growth.

Czapek (**5**) in 1902 performed some of the earliest experiments in which compounds related to the nucleic acid derivatives were tested as sources of nitrogen for fungi. Allantoin, alloxan, urea and uric acid were shown to be suitable nitrogen sources for *Aspergillus niger*, while barbituric acid and caffeine were less favorable for growth. Iwanoff (**10**) grew *A. niger* upon a medium containing thymus desoxyribonucleic acid as the sole source of nitrogen. After growth for 32 days, two-thirds of the nitrogen originally provided as nucleic acid was recovered in the mycelium of the fungus.

Kossowicz (**12**) cultivated *Aspergillus glaucus*, *A. niger*, *Botrytis bassiana*, *Cladosporium herbarum*, *Isaria farinosa*, *Penicillium glaucum*, *P. brevicaule*, *Phytophthora infestans*, *Mucor* sp., and *Fusarium* sp. upon media containing urea, uric acid, guanine, or guanidine·HCl as the nitrogen source, finding that all of these organisms grew well upon each of the compounds tested.

Brunel (**2**) found that uric acid and allantoin were satisfactory substrates for the growth of a large number of species of Basidiomycetes, including representatives of the Agaricaceae, Boletaceae and Polyporaceae. Employing enzymatic methods, he demon-

strated the conversion of uric acid to allantoin, allantoin to allantoic acid, and allantoic acid to glyoxylic acid and urea, by the enzymes uricase, allantoinase, and allantoicase respectively. In subsequent studies with *Sterigmatocystis nigra* (Brunel, **3**, **4**) it was shown that allantoinase, allantoicase, and urease were constitutive enzymes in this organism, while uricase was not formed under any cultural condition. Recently Franke, Taha and Krieg (**9**) have made extensive studies of uric acid decomposition by a number of Phycomycetes (including species of *Rhizopus, Mucor* and *Cunninghamella*); Ascomycetes (including a number of species of *Aspergillus, Penicillium* and *Neurospora sitophila*); and Fungi Imperfecti (principally species of *Alternaria* and *Fusarium*), concluding that uricase is almost universally present as a constitutive enzyme among fungi.

Di Carlo, Schultz and McManus (**6**) have tested a large number of nucleic acid derivatives as nitrogen sources for the growth of the yeasts, *Saccharomyces cerevisiae* and *Torula utilis*. *Torula utilis* was able to grow upon media containing adenine, adenosine, adenylic acid, guanine, guanosine, guanylic acid, cytosine, uracil, xanthine, xanthosine, hypoxanthine, uric acid, or allantoin. It did not, however, utilize cytidine, cytidylic acid, uridine, uridylic acid, thymine, alloxan, ribonucleic acid or desoxyribonucleic acid. Thus the purine nucleosides and nucleotides were assimilated, while the pyrimidine nucleosides and nucleotides were not. *Saccharomyces cerevisiae* was able to utilize only adenine, guanine, cytosine and allantoin, of the substances tested, and thus was far less versatile than *Torula utilis*.

Kerr, Seraidarian and Brown (**11**) have studied the utilization of purines, purine nucleosides and nucleotides for ribonucleic acid synthesis in *Torulopsis utilis*, using precursors labeled with C^{14}. It was found that guanine could be formed from adenine, but this reaction was not reversible. While adenine and guanine were almost completely incorporated into ribonucleic acid, their nucleosides and nucleotides were utilized far less extensively for ribonucleic acid synthesis.

It is the purpose of the present work to study the utilization of nucleic acid derivatives and related compounds by the fungus *Neurospora sitophila*.

MATERIALS AND METHODS

The organism used in these experiments was a wild-type strain of *Neurospora sitophila*, "Beall" race, mating type A, originally obtained from Dr. B. O. Dodge, New York Botanical Garden (Dodge, **7**). The basal medium used throughout the work was a modification of the minimal medium of Beadle and Tatum (**1**), in which ammonium tartrate and ammonium nitrate were omitted. Its composition was as follows:

KH_2PO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
NaCl	0.1 gm
$CaCl_2$	0.1 gm
Sucrose	15.0 gm
Biotin	5 μ gm

Trace elements: Bo, 0.01 mg; Cu, 0.1 mg; Fe, 0.2 mg; Mn, 0.02 mg; Mo, 0.02 mg; Zn, 2.0 mg
Distilled water, to 1 liter

To this basal medium were added various nucleic acid derivatives in amounts calculated to provide 50 mg nitrogen per 100 ml. The pH was adjusted to 5.5, and the media were sterilized by autoclaving.

The media were inoculated with spores obtained from stock cultures grown on Beadle and Tatum's minimal medium. Cultures were grown in 125 ml Erlenmeyer flasks containing 25 ml of medium. All experiments were done in quadruplicate. After growth for 10 days at 30° C, the mycelia were removed by filtration, dried to constant weight in a desiccator over drierite, and weighed. In a few instances, it was necessary to repeat the experiments in order to obtain satisfactory agreement among the four replications. Controls were included containing no nitrogen, sodium nitrate, sodium nitrite, ammonium sulfate or asparagine as the nitrogen source, in order to facilitate comparison with the results obtained with nucleic acid derivatives.

The pH of all culture filtrates was determined at the end of the growth period. In all cases, except for ammonium sulphate and guanidine hydrochloride, the pH exhibited a trend toward greater alkalinity during growth, with final values ranging from 5.5 to 7.5.

The sources of the experimental chemicals were as follows: Orotic acid, thiouracil, thymine, hypoxanthine, theophylline, benzimidazole, adenosine, cytidine sulfate, inosine, uridine, xanthosine, adenylic acid, cytidylic acid, guanylic acid, and uridylic acid were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Uracil, adenine sulfate, guanine hydrochloride, xanthine, allantoin, guanosine, ribonucleic acid and desoxyribonucleic acid were obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

Alloxan; barbituric acid; 2-methyl, 5-ethoxymethyl, 6-amino pyrimidine; uric acid; caffeine and urea were obtained from Merck and Company, Inc., Rahway, N. J. Cytosine, 5-methyl cytosine and 2,6-diaminopurine were obtained from Dougherty Chemicals, Richmond Hill, N. Y. Guanidine hydrochloride was obtained from Eastman Kodak Company, Rochester, N. Y., and 8-azaguanine was obtained from the Southern Research Institute, Birmingham, Ala.

TABLE I

GROWTH OF NEUROSPORA SITOPHILA IN MEDIA CONTAINING VARIOUS NUCLEIC ACID DERIVATIVES AS SOLE SOURCES OF NITROGEN

Nitrogen source	Mat weight
CONTROLS	
No nitrogen	0 mg
Sodium nitrate	112 ± 8
Sodium nitrite	0
Ammonium sulphate	23 ± 1
Asparagine	136 ± 6
PYRIMIDINES	
Alloxan[1]	0
Barbituric acid	6 ± 2.5
Cytosine	4 ± 1.5
5-methyl cytosine	5 ± 1
Orotic acid	6 ± 1
2-methyl, 5-ethoxymethyl, 6-amino pyrimidine	11 ± 2
Thiouracil[2]	13 ± 2
Thymine	4 ± 1.5
Uracil	4 ± 1.5
PURINES AND RELATED COMPOUNDS	
Adenine sulphate	82 ± 13
Guanine hydrochloride[2]	85 ± 5
Hypoxanthine	137 ± 12
Xanthine[2]	122 ± 16
Uric acid[2]	132 ± 12
Theophylline	12 ± 2
Caffeine	9 ± 1
Allantoin	119 ± 7
Urea	147 ± 5
Guanidine hydrochloride	42 ± 8

TABLE I—*Continued*

Nitrogen source	Mat weight
PURINE INHIBITORS	
Benzimidazole	0
2,6-diaminopurine[2]	0
8-azaguanine[2]	15 ± 3.5
NUCLEOSIDES	
Adenosine	130 ± 1
Cytidine sulphate	48 ± 12
Guanosine	133 ± 6
Inosine	137 ± 4
Uridine	14 ± 3
Xanthosine	134 ± 12
NUCLEOTIDES	
Adenylic acid	116 ± 3
Cytidylic acid	86 ± 9
Guanylic acid	127 ± 10
Uridylic acid	18 ± 9
NUCLEIC ACIDS	
Desoxyribonucleic acid[2]	105 ± 6
Ribonucleic acid[2,4]	106 ± 13

[1] Decomposed on autoclaving.
[2] Insoluble in the concentration used.
[3] The quantity used was calculated on the basis of 16.7% nitrogen content.
[4] The quantity used was calculated on the basis of 16.1% nitrogen content.

RESULTS

The results of the various experiments are presented in TABLE I, in terms of the weights of mycelia produced in media containing various nucleic acid derivatives as sole sources of nitrogen, in quantities equivalent to 50 mg nitrogen per 100 ml. In order to give some indication as to the reliability and reproducibility of the data, the standard deviations have been calculated from the individual mat weights found in the four replications of each experiment.

Because of the possibility of breakdown of nucleosides and nucleotides upon autoclaving, an experiment was done in which adenosine and adenylic acid were sterilized by passage through a Mandler filter, and added to basal medium which had been autoclaved. Under these conditions, mat weights obtained in adenosine averaged 134 mg as against 130 mg obtained on autoclaving, and with adenylic acid the values were 120 mg and 116 mg respectively. It was assumed, therefore, that destruction of other nucleosides and nucleotides upon autoclaving was negligible.

Since the studies of Woolley (**20**) had indicated that benzimidazole was a competitive inhibitor of purines in experiments with yeast, experiments were performed to test this substance as an inhibitor of purine utilization, with *Neurospora* as the test organism. In these experiments, hypoxanthine was used as the nitrogen source, at a concentration of 122 mg per 100 ml (corresponding to 50 mg percent of nitrogen), and the concentration of

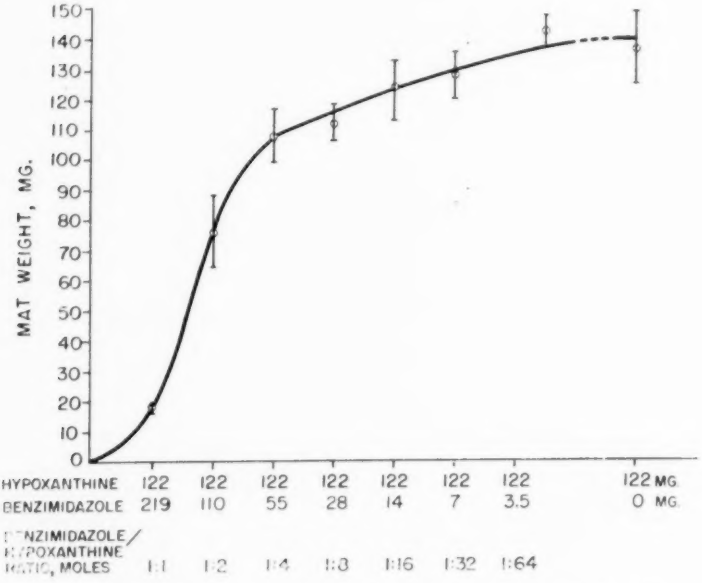

FIG. 1. The effect of varying amounts of benzimidazole upon the growth of *Neurospora sitophila*.

benzimidazole was varied. The results are presented in FIG. 1, in which each point represents the mean of four determinations, and the standard deviations are indicated by the lengths of the vertical lines passing through each point.

It may be noted that the growth of *N. sitophila* was almost completely inhibited by a benzimidazole/hypoxanthine ratio of 1:1, while the growth resulting from ratios of 1:16, 1:32 or 1:64 closely approximates that in the controls with hypoxanthine alone.

Fifty percent inhibition of growth was obtained at a benzimidazole/hypoxanthine ratio very close to 1 : 2.

DISCUSSION

In order to provide a standard of comparison for the nutritive values of the various nucleic acid derivatives for *Neurospora*, cultures were grown in media containing no nitrogen, or with sodium nitrate, sodium nitrite, ammonium sulphate, or asparagine as the nitrogen source. No growth occurred in the absence of nitrogen, or in sodium nitrite, the nitrite proving to be toxic in the concentration used. Growth was poor in ammonium sulphate because of the lowering of the pH of the medium to 3.8, a level unfavorable for growth. Sodium nitrate and asparagine proved to be excellent nitrogen sources for *N. sitophila*.

Pyrimidines proved to be very poor as nitrogen sources for *Neurospora*. Only a very small amount of growth occurred in the nucleic acid components cytosine, 5-methyl cytosine, thymine, or uracil. Barbituric acid, which has been recently shown to be a normal intermediate in pyrimidine metabolism, arising upon uracil oxidation by an unidentified soil bacterium (Wang and Lampen, **19**) and by *Nocardia corallina* (Lara, **13**, **14**), supported only a trace of growth. Orotic acid has been shown to be able to substitute for uracil as a growth factor for a pyrimidineless mutant of *Neurospora crassa* and thus function as a uridine precursor (Mitchell and Houlahan, **16**), to be accumulated as a metabolic product in another pyrimidineless mutant of this species (Mitchell, Houlahan, and Nyc, **17**), and to be incorporated into the ribose nucleoside, orotidine (Michelson, Drell and Mitchell, **15**). It proved to be a poor nitrogen source for *N. sitophila* in the present experiments, as did alloxan, thiouracil, and the pyrimidine moiety of thiamine.

In contrast to the pyrimidines, purines were excellent nitrogen sources for *N. sitophila*. Good or excellent growth was obtained on adenine, guanine, hypoxanthine, xanthine, and uric acid. It is apparent from the data presented that a significant difference exists between adenine and guanine, the purine components of nucleic

acids, and the remaining purines tested, adenine and guanine being only about two thirds as effective nitrogen sources as hypoxathine, xanthine, or uric acid. The methylated purines, theophylline and and caffeine, are poor sources of nitrogen.

Allantoin and urea were excellent nitrogen sources for *N. sitophila*. The utilization by this fungus of adenine, guanine, hypoxanthine, xanthine, uric acid, allantoin and urea indicates the presence in this organism of the enzymes adenase, guanase, xanthine oxidase, uricase, allantoinase and urease. These findings suggest that the classical scheme of purine metabolism is operative in *N. sitophila*.

Three purine antagonists were studied; namely, benzimidazole, 2,6-diaminopurine and 8-azaguanine (guanazolo). No growth occurred in media containing benzimidazole or 2,6-diaminopurine as the nitrogen source, while slight growth was obtained upon 8-azaguanine. Drell (**8**) has recently reported that 2,6-diaminopurine, in relatively low concentrations, completely inhibits growth of a purineless mutant of *Neurospora crassa* in the presence of adenine or hypoxanthine, but not in the presence of adenosine or inosine. No previous work with benzimidazole or 8-azaguanine in connection with *Neurospora* has come to the writer's attention.

The data reported in FIG. 1 indicate that 50% inhibition of growth of *N. sitophila* was obtained at a benzamidazole concentration of 1.1 mg per ml. Woolley (**20**) reported half maximal inhibition of *Saccharomyces cerevisiae* at a benzimidazole concentration of 0.3 mg per ml. Further, from the data presented in FIG. 1, it may be noted that the benzimidazole/hypoxanthine molar ratio corresponding to 50% inhibition of growth of *N. sitophila* is approximately 1:2, indicating an inhibition index of 0.5.

It is further apparent that *N. sitophila* utilizes certain of the purine nucleosides and nucleotides for growth to a greater extent than it does the corresponding free purines. The amount of growth obtained upon adenosine, adenylic acid, guanosine or guanylic acid are significantly higher than those obtained upon adenine or guanine. Pierce and Loring (**18**) have concluded that the nucleosides play a more central role in nucleic acid metabolism in *N. crassa* than either the nucleotides or the free bases.

The differences in growth-promoting activity between adenine or guanine and their ribosides are not found to occur between hypoxanthine and inosine, nor between xanthine and xanthosine. It would appear to be of some significance to note that adenine and xanthine are constituents of the nucleic acids, while hypoxanthine and xanthine are not.

Among the pyrimidine nucleosides and nucleotides tested, little difference was found to occur among uracil, uridine or uridylic acid, all of which were poor nitrogen sources for *N. sitophila*. Striking differences were noted, however, among the cytosine, cytidine, cytidylic acid series, growth in cytidylic acid being 20-fold greater than in cytosine, and comparable to that obtained in adenine or guanine.

These experiments have been limited to ribosides and ribotides, and include none of the desoxyribose derivatives except for desoxyribonucleic acid. Desoxyribonucleic acid and ribonucelic acid are both utilized by *N. sitophila* as a nitrogen source, with equal effectiveness. As might be anticipated, the amount of growth resulting from these materials is intermediate between the high values obtained with certain purines and the low values obtained with pyrimidines.

The findings would indicate that *N. sitophila* is considerably more versatile, in its utilization of nucleic acid derivatives, than is either of the two yeasts studied by Di Carlo, Schultz and McManus (**6**).

ACKNOWLEDGMENTS

This study has been supported by grants from the Mycological Society of America and from the Natural Science Research Fund of Vanderbilt University, which provided for the chemicals used. The writer wishes also to acknowledge the donation of 2-methyl, 5-ethoxymethyl, 6-amino pyrimidine by Merck and Company, Rahway, N. J., and 8-azaguanine, which was contributed by the Southern Research Institute, Birmingham, Ala. The assistance of Mr. William T. Allman, Jr., in this project was made possible through a fellowship of the General Education Board.

SUMMARY

The utilization of 34 nucleic acid derivatives and related compounds has been studied in *Neurospora sitophila*. All of the pyrimidines tested (cytosine, 5-methyl cytosine, thymine, uracil, orotic acid, barbituric acid, alloxan, thiouracil, and the pyrimidine moiety of thiamine) were very poor nitrogen sources for growth of this fungus. The purines adenine, guanine, hypoxanthine, xanthine and uric acid, as well as allantoin and urea were excellent nitrogen sources for *N. sitophila*. Growth was poor upon the methylated purines, caffeine and theophylline.

N. sitophila utilizes certain of the purine and pyrimidine nucleosides and nucleotides (adenosine, adenylic acid, guanosine, guanylic acid, cytidine, cytidylic acid) for growth to a greater extent than is the case with the corresponding free bases. Comparable differences were not found between hypoxanthine and inosine, or between xanthine and xanthosine, all of which were excellent as sources of nitrogen for growth, nor among uracil, uridine and uridylic acid, which were poor nitrogen sources. Both ribonucleic acid and desoxyribonucleic acid are favorable nitrogen sources for growth of *N. sitophila*.

N. sitophila is unable to utilize the purine antimetabolites benzimidazole or 2,6-diaminopurine as a source of nitrogen, while 8-azaguanine is a poor nitrogen source.

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A FUSIDIUM DISEASE OF XYLARIA IN WISCONSIN[1]

M. P. Backus and E. A. Stowell

(WITH 10 FIGURES)

An infectious disease affecting stromata of *Xylaria* has been encountered by the writers in the course of studies on various representatives of the Xylariaceae in southern Wisconsin. A single species of *Xylaria* has been found attacked in nature and in only one location, but the disease attained epidemic proportions in this situation. It first came to notice in the spring of 1951, although later examination of preserved specimens taken from the same site on previous occasions revealed that the pathogen was present here as early as 1945. The malady appeared to be highly destructive in 1951, and another local epidemic developed during wet weather in early June, 1952. It has been determined that the disease is incited by a fungus, *Fusidium parasiticum* Westendorp, which appears not to have been previously reported in North America.

THE HOST

The malady in question was encountered in a heavy stand of stromata of a species of *Xylaria* identified by Professor Julian Miller as *X. oxyacanthae* Tul. This is a species concerning which there has been considerable confusion and debate. Lloyd (**4**), Gilman and Tiffany (**2**), and others have considered it synonymous with *X. carpophila* (Pers.) Fr. The stand of stromata was located beneath a clump of hawthorns (*Crataegus mollis* ?) on the University of Wisconsin campus. In this location the *Xylaria* was first noted about 1944 and has reappeared each succeeding spring since that time. The stromata in this species are small,

[1] The work was supported by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

often flattened, and frequently digitately branched above. Each stroma dug up by the writers has been found to be growing out of a buried hawthorn "seed" (nutlet), from which the mycelium apparently draws the major portion of its nourishment. In the vicinity of Madison, Wisconsin, the stromata of *X. oxyacanthae* usually make their appearance near the end of May. In 1951 there were many thousands of them present in an area of perhaps two square rods in the small hawthorn grove referred to above. Although slender and only about an inch high, the young stromata are conspicuous because of their light cream color; in large numbers, standing in close array, they present at this stage an arresting sight. After the production of conidia ceases, the stromata become dull grey, blending with the soil. The stromata of *X. oxyacanthae* make their appearance earlier in the growing season than do those of any of the larger Xylarias which are encountered in southern Wisconsin, and maturation of their ascocarps is more rapid. In about six weeks after the first appearance of the fungus above the soil, mature ascospores are present.

SYMPTOMS AND PROGRESS OF THE DISEASE

About the second week in June, 1951, small pure white spots were observed on a number of stromata which had been brought into the laboratory. These were full-grown stromata in which ascocarp development was under way and in which conidial production had declined. The surface of the stroma was becoming gray at this stage, and the white spots observed stood out prominently. Under a lens they appeared downy, resembling patches of downy mildew conidiophores; and microscopic examination revealed that they consisted, indeed, of dense masses of conidiophores and spores of an extraneous fungus. The number of stromata showing such patches soon increased until, particularly at one end of the stand, a very large proportion showed them. Furthermore, lesions of greater extent were now often encountered, some of them involving a large section of the affected stroma (FIG. 1). As the disease spread, it was noted that the *Xylaria* population declined, and close examination revealed many collapsed and withered stromata, shrunken down close to the soil

surface. By early July scarcely a hundred stromata could be found on the site. These remaining few were, however, firm and erect; and they contained mature asci.

The disease was slightly less severe in 1952, but its general

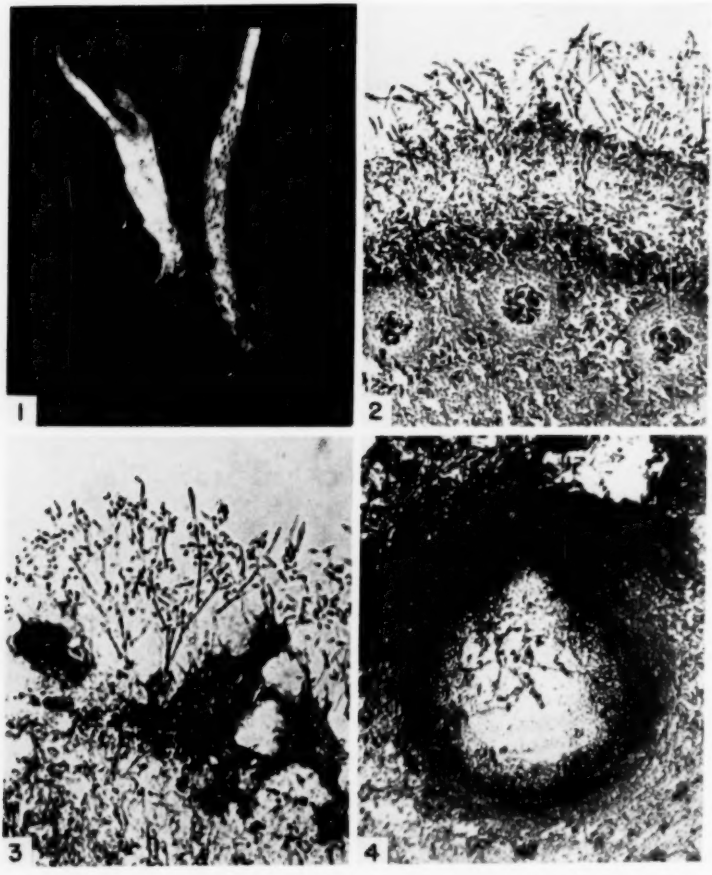


FIG. 1. Infected stromata of *Xylaria oxyacanthae* with white downy patches of *Fusidium* conidiophores, × 2. FIG. 2. Section through diseased stroma, showing conidiophores and invading mycelium of the parasite, × 165. FIG. 3. Branching conidiophores of *Fusidium* growing out from the conidial layer of the host. The dark areas represent masses of bacterial cells, × 195. FIG. 4. Aborted perithecium invaded by parasitic mycelium, × 170.

course was the same. In 1952 there was an early spring in Wisconsin, and the *X. oxyacanthae* stromata emerged nearly two weeks earlier than usual. Alerted to the possibility of a recurrence of the disease that had been prevalent the previous year, the writers kept close watch for the appearance of the characteristic stroma lesions; the first one was found on May 21. Thereafter the disease spread rapidly, and by the first week in June infected stromata were distributed throughout most of the stand. Again the *Xylaria* population rapidly declined, although the progress of the disease seemed to be temporarily arrested during a period of warmer weather which occurred about mid-June.

In *X. oxyacanthae* a certain proportion of the stromata are normally sterile, and these wither early, infected or not; but the impression was inescapable that in 1951 and 1952 the decimation of the *Xylaria* population in question was associated with a virulent infection. In the same location several years earlier thousands of stromata had been observed to come to maturity.

PATHOGONICITY STUDIES

The fungus forming white patches on the *X. oxyacanthae* stromata was successfully isolated by streaking conidia onto plates of 4 percent water agar and then picking out individual germinating spores with a sterile microscalpel under a high-powered stereoscopic microscope. Growth of this fungus on agar is very slow, but eventually some tube cultures were obtained which provided inoculum for pathogenicity tests.

Pure cultures of *X. oxyacanthae*, *X. polymorpha* (Pers.) Grev., *X. cornu-damae* (Schw.) Berk., and *X. acuta* Peck (?)[2] were available in the culture collection of the University of Wisconsin Department of Botany, these having been isolated several years previously by one of the writers. From them, cultures in cotton-plugged 500 ml Erlenmeyer flasks were now prepared. Previous experience in this laboratory having shown that autoclaved wheat grains provide a substrate favorable for stroma development, this medium was adopted for the flask cultures set up. The cultures

[2] The authors are indebted to Professor Julian Miller for this tentative identification.

were incubated on a laboratory shelf in diffuse light, since light had also been shown to be important for good development of stromata.

When the *Xylaria* stromata had reached nearly maximum size in the flasks, they were inoculated either by spraying them with a suspension of spores from tube cultures of the suspected pathogen, or by placing in contact with certain stromata small blocks of agar on which this organism was growing. The tests were repeated several times over a period of about nine months, two to four flask cultures of each of the four *Xylaria* species being included in each test.

In no case was infection of stromata of *X. cornu-damae* or *X. acuta* (?) accomplished. In a few instances very limited infection of stromata of *X. polymorpha* occurred in flasks inoculated by spraying. With *X. oxyacanthae*, infection was obtained by both methods of inoculation employed and was considerably more abundant than in the case of *X. polymorpha*. Typical white downy patches developed on the affected stromata, and the parasite was successfully reisolated.

On the whole, however, even in the case of the *X. oxyacanthae* cultures, infection was relatively sparse and the progress of the disease slow compared with that observed in nature. Various factors conceivably may have been limiting in the tests carried out. Only very light spore suspensions could be prepared from the scanty cultures which had been grown to provide inoculum; undoubtedly heavier conidial suspensions would have been desirable. Moisture conditions in the flasks very probably were not ideal; and the inoculations might have been more successful had the host been wounded, since some evidence has accumulated indicating that wounds may favor the entrance of the pathogen. However, the factor which very easily could have been the most important of all was the condition of the host. Stromata developed in cultures of the type used are far from normal. They are highly succulent; conidial production is sparse, and few if any ascocarps are initiated. Furthermore, the stromata do not, in appearance, closely resemble those produced in nature. For the most part they are much smaller and may not have the form characteristic of the species.

Curiously enough, those of *X. oxyacanthae* are typically larger than *X. oxyacanthae* stromata naturally produced; but they, too, are more or less abnormal in shape, and furthermore they usually develop in such a dense mass that their bases are in close contact or even fused.

HISTOLOGICAL OBSERVATIONS

Diseased stromata, collected in the field during both the 1951 and 1952 epidemics, were fixed in formol-acetic-alcohol and imbedded in paraffin. Microtome sections were stained with Heidenhain's iron-alum haematoxylin.

In the finished preparations, the mycelium of the parasite was observed growing throughout the stromata. The most profuse development appeared to be in the area of the conidiophore layer of the host, but hyphae were also commonly seen ramifying into the interior of the stroma among the massed hyphae of the host. They are readily distinguished from the *Xylaria* stromatic hyphae, primarily because they stain more heavily and because their nuclei are more prominent. In addition, the parasitic hyphae are generally of slightly greater diameter, and their orientation differs from that of the *Xylaria* filaments, most of which run parallel to the long axis of the stroma.

Interestingly enough, in the light of the field observations, most of the preparations examined showed surprisingly little damage to the host except in the area outside the ectostroma. Probably bearing on this situation is the fact that the specimens selected for fixation were mainly some which showed the conidiophores of the parasite in best condition; and it would now appear that the chief damage to the interior of the stroma must occur later. In any case there was little evidence of discoloration, shriveling, dissolution, or other degenerative changes of the ordinary stromatic hyphae in the region penetrated by the hyphae of the parasite. Furthermore, many instances were noted where the "Woronin's hyphae" of the young ascocarp initial appeared still to be in a healthy condition, even though numerous hyphae of the parasite were very close by. Occasionally normal and deteriorating initials were found immediately adjacent to one another. Thus, in FIG.

2 the dense uniform staining of the cells in the coils within the two initials at the left clearly indicates degeneration, while in the initial at right normal-appearing nuclei and cytoplasm could be observed in the more faintly staining fertile hyphae. Since in any healthy stroma a certain portion of the ascocarp initials regularly abort, it is difficult in the case of the parasitized stroma to be sure that any given degeneration represents damage caused by the fungal invader. Still, counts made in diseased specimens and in healthy specimens of approximately the same age do indicate that the number of aborting initials is considerably higher in the former. Furthermore, at a somewhat later stage, hyphae of the parasite have been identified actually growing through the interior of deteriorating young perithecia (FIG. 4) which had reached a point in development beyond that at which ordinary abortion usually occurs.

Various observations suggest that the ectostroma of the host is a barrier to the inward movement of the mycelium of the parasite. This stromatic layer, which is located a short distance below the *Xylaria* conidiophores, is composed of dark, closely-interwoven thick-walled hyphae. It is, however, a zone of rather uneven thickness and varying compactness. It may be that the parasite penetrates it only at its weakest points. Some small lesions have been noted where the mycelium appeared to be entirely exterior to this barrier; and it is quite possible that in some instances the pathogen never does succeed in breaking through into the interior of the host. Under natural conditions slugs, insects, etc. may cause breaks in the ectostroma, and such wounds undoubtedly assist the pathogen in getting into the interior of the stroma in some cases. Deep-seated infections have been noted in connection with some of these openings. The parasite can also gain easy entrance into the interior in another way, for infection may occur in the young upper portion of the stroma where the ectostroma is not yet differentiated.

Bacterial pockets (FIG. 3) have quite often been found associated with the fungal lesions. These pockets are sometimes of considerable size and may extend some distance into the entostroma. Conidiophores and stromatic hyphae of the *Xylaria* are

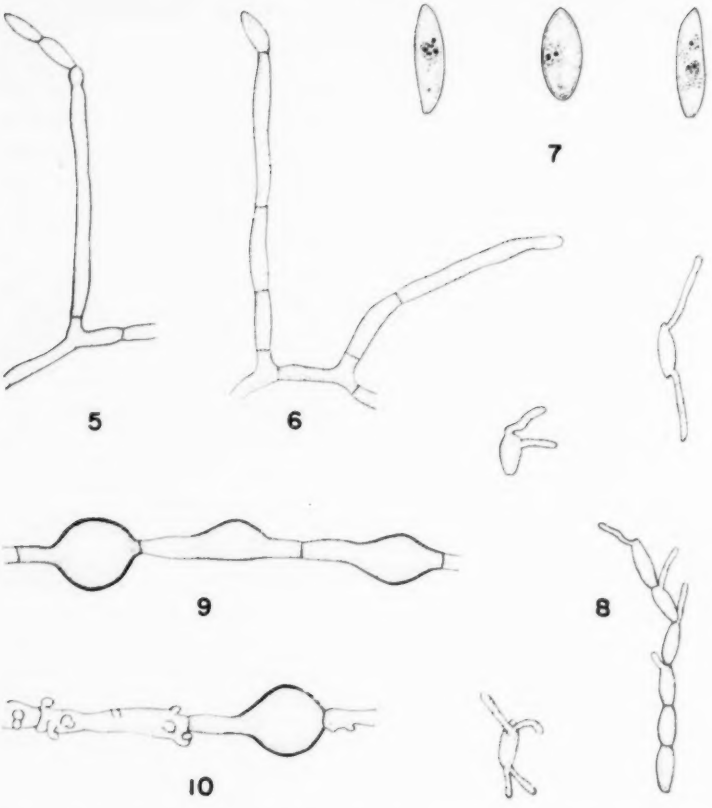
obviously dissolved by bacterial action in these areas, and the prepared slides reveal a solid mass of rod-type bacterial cells filling the lysigenous cavities. Since bacterial pockets have rarely been encountered except in association with the fungal lesions, it seems that the fungal parasite must somehow open the way for the bacterial invader. Wounds of the type which appear to facilitate entrance of the fungus into the stroma interior may also assist the bacteria in establishing themselves deep in the host tissue.

Fusidium conidiophores are produced in great abundance at the surface of the infected stroma, extending well beyond the shorter conidiophores of the host (FIGS. 2, 3). In microtome sections the conidiophores of the parasite were observed to be simple or sparingly branched, relatively coarse structures bearing unicellular fusoid conidia at their tips. The conidia (FIG. 7) and the cells of the conidiophores, like the mycelial cells, are uninucleate.

CULTURAL STUDIES

The fungal parasite was found to grow readily but very slowly on corn meal agar and on a *Xylaria* decoction agar. The latter medium was prepared using the extract of 25 gms of *X. oxyacanthae* stromata, 10 gms of dextrose, and 20 gms of agar per liter. On the corn meal agar, colonies showed an appressed, spreading growth reaching a diameter of slightly less than 2 cm in four weeks with incubation at 20° C. Conidiophores formed were mainly unbranched (FIGS. 5, 6) and were generally much shorter than those developed on the host, averaging only about 80 μ in length. They grew erect and bore long chains of hyaline, fusoid conidia. On the *Xylaria* decoction agar the growth was raised and cottony, but colonies 3–4 weeks old had a diameter of only a few millimeters. Here, too, however, spores were produced in moderate abundance. Conidia formed in culture were more or less spindle-shaped, averaging 11 × 3.6 μ. They corresponded closely in size and shape with conidia produced in nature, but much longer chains of spores were observed in the cultures than were ever found on diseased *Xylaria* specimens.

This fungus developed very poorly on a number of other common laboratory media on which an attempt was made to cultivate



FIGS. 5, 6. *Fusidium* conidiophores bearing conidia, from corn meal agar cultures, × 550. FIG. 7. Conidia, stained to show cytological detail, × 1100. FIG. 8. Germinating conidia, × 550. FIG. 9. Developing chlamydospores, from corn meal agar cultures, × 550. FIG. 10. Hypha with chlamydospore and with out-pocketings characteristic of growth on mannite agar, × 550.

it. Potato-dextrose, mannite, corn steep, honey-peptone, and Czapek's agars all proved to be unsatisfactory substrates. No production of conidia was obtained on any of these, and mycelial growth, when, indeed, it could be established at all, was extremely meager and abnormal. On the mannite agar [3] hyphal cells regu-

[3] Per liter: beef extract 3.0 gms; peptone 5.0 gms; mannitol 10.0 gms; agar 15.0 gms.

larly produced dwarf branches in the form of curious outpocketings (FIG. 10). These peculiar outgrowths have also been seen in cultures on corn meal agar and on *Xylaria* decoction agar, but much less frequently.

On most media used, chlamydospores were produced in the vegetative hyphae (FIG. 9); and these were especially abundant on the mannite agar. Similar spores have in rare instances been encountered in the hyphal mass from which the conidiophores arise on the *Xylaria* stromata. It is possible that they may play a part in carrying the fungus over winter.

Conidia produced in culture and conidia removed from diseased *Xylaria* stromata germinate readily on water agar and in hanging drops of distilled water. As a rule, one or two (rarely 3 or 4) germ tubes are produced. Spores from cultures may even germinate while still in chains (FIG. 8).

TAXONOMY

As noted above, the *Xylaria* pathogen in question appears not to have been previously reported in this country; but perusal of the foreign literature has revealed that an imperfect fungus closely resembling the Wisconsin organism is known in Europe.

Westendorp (**7**) in 1851 described *Fusidium parasiticum* from a collection or collections made by him on *Xylaria cornuta* in Belgium. He gave a brief but good description of the parasite and its appearance on the host; and he further recorded the observation that *Xylaria* stromata infected with this parasite almost never succeeded in coming to maturity. Spores of the *Fusidium* were described as about 1/50 mm long by 1/200 mm wide ($= 20 \times 5\,\mu$). Some forty years later Lambotte (**3**) reported another collection of this fungus on *X. cornuta* in Belgium. Additional collections from Belgium were recorded by De Wildeman (**1**) in 1898 with no description or comments included.

A similar fungus on *X. cornuta*, collected near Noidan, France (Côte d'Or) by F. Fautrey in 1890, is included as *No. 5795* in Roumeguère's "Fungi Gallici Exsiccati". In the published account of fungi comprising Centurie LVIII (**6**), this organism is listed as *Fusidium parasiticum* West. forma *microconidia*, with the

notation "(10,12 × 3,3½ au lieu de 20 × 5)". It is assumed that these figures represent the dimensions, in microns, of the conidia of this parasite as contrasted with the measurements given by Westendorp in his original description of the species. Oudemans (5) lists this parasite on *X. cornuta* as *Fusidium parasiticum* West. forma *microconidia* Fautr., ascribing the form name to the man who collected the fungus in 1880 and who apparently prepared the notes on it for the above-cited French article.

Since Westendorp's observations were made at an early date, it seems possible that the measurements which he gave for the conidia of his *Xylaria* parasite could have been erroneous, and that Fautrey's fungus was really identical with the one described from Belgium on the same host. The Wisconsin material seems to correspond closely with the general description provided by Westendorp, but the size of the conidia coincides almost exactly with the measurements given for the fungus collected by Fautrey.

Specimens of *Fusidium parasiticum* on *Xylaria oxyacanthae* have been deposited in the University of Wisconsin Herbarium, the Farlow Herbarium of Harvard University, and in the Mycological Collections of the U. S. Dept. of Agriculture Bureau of Plant Industry; also in the cryptogamic herbaria of the University of Toronto, University of Michigan, University of California, University of Wyoming, and Washington State College.

SUMMARY

Fusidium parasiticum Westendorp, apparently previously unreported in the United States, has been found inciting a destructive disease of *Xylaria oxyacanthae* stromata at Madison, Wisconsin.

The fungus appears in late spring in the form of white, downy mildew-like lesions on the *Xylaria* stromata. As the disease progresses, the infected stromata become shrunken and disintegrate without maturing ascospores.

The fungus has been isolated and studied in pure culture. Of the various media on which cultivation of the organism was attempted, corn meal agar and *Xylaria* decoction agar supported the best mycelial growth, and these were the only substrates upon which sporulation was obtained *in vitro*.

Flask cultures of four Wisconsin species of *Xylaria* were inoculated with spore suspensions and mycelium from agar culture of *Fusidium parasiticum*. Moderate infection of the *X. oxyacanthae* stromata was obtained, and the parasite was reisolated from the lesions on the host.

In microtome sections through diseased *Xylaria* specimens the most profuse development of the *Fusidium* mycelium was observed to be in the area outside the ectostroma, but hyphae may penetrate to the very center of the host.

Bacterial pockets are commonly found associated with the *Fusidium* lesions. The bacteria involved are interpreted as secondary invaders.

Initial damage to the interior of the stroma is mild, even when the mycelium has penetrated deeply. There is, however, a marked tendency toward ascocarp abortion in later stages of infection.

The parasite produces septate, sparingly-branched conidiophores which bear long chains of hyaline, fusoid conidia. Haematoxylin-stained preparations revealed conidia, conidiophore cells, and cells of the mycelium to be uninucleate.

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THE DIAMETER OF THE MOLD COLONY AS A RELIABLE MEASURE OF GROWTH [1, 2]

F. P. Brancato [3] and N. S. Golding

(WITH 6 FIGURES)

In an investigation in which the effect of certain controlled factors on the growth of an organism is to be considered, it is essential that the range of sensitiveness of the phenomenon chosen as an indicator of this influence be known. The diameter of the fungus colony has often been used as a measure of growth. In contrast, only a comparatively few investigators have attempted to evaluate this method of measuring fungus growth (Brown, **1922, 1923**; Crozier, **1926**; Worley, **1939**). The object of this study was to determine the individual influence of some important environmental factors on the diameter attained by colonies of several common molds within a definite period of time, viz. rate of growth.

MATERIALS AND METHODS

The organisms used in this study were *Aspergillus niger* van Tieghem, *14*, and *A. flavus* Link, d; *Penicillium notatum* Westling, NRRL *832*, *P. expansum* Link, *69*, and *P. roqueforti* Thom, *33*; and *Geotrichum candidum* Link ex Loubière, m.[4]

Malt agar (Difco) was used as the basal medium. The des-

[1] Scientific Paper No. 1138, Washington Agricultural and Experiment Stations, Pullman. Project No. 1034.

[2] This investigation was supported in part by funds provided for biological and medical research by the State of Washington, Initiative Measure 171.

[3] Data from thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology, Department of Bacteriology and Public Health, The State College of Washington. Present address is the United States Public Health Service Hospital, Seattle, Washington.

[4] Strains stored in the culture collection of the Department of Dairy Science, The State College of Washington, Pullman, Washington, under the code figures or letters following their names.

sicated medium was mixed with the required amount of distilled water and heated until completely dissolved. Twenty-five ml aliquots of this hot fluid medium were measured into 125 ml Erlenmeyer flasks by means of an autonomic delivery pipette. These flasks were autoclaved at 15 pounds pressure for 15 minutes (119° C). They were then cooled slightly and poured into sterile petri plates. The pouring was always performed within an inoculation chamber to minimize contamination.

The inocula for the agar plates were provided in the following manner: One ml of sterile distilled water was withdrawn from a 100 ml quantity and added to a 10-day-old malt agar slant culture. After mixing, as much of the water as possible was withdrawn and returned to the water blank. An L-shaped platinum wire was dipped into the resulting spore suspension. The center of the plated medium was pierced carefully with the tip of this moistened wire.

Unless otherwise specified, *A. niger* was incubated at 35° C for 3 days, *A. flavus* at 32.2° C for 4 days, *G. candidum* at 29.4° C for 7 days, *P. notatum* at 26.7° C for 7 days, *P. roqueforti* at 23.9° C for 7 days, and *P. expansum* at 21.1° C for 7 days. Except in the experiments on relative humidity, saturated aqueous solutions of $(NH_4)_2SO_4$ maintained the relative humidity of the atmosphere in the incubation chamber at about 80 percent.

The average diameter in mm of the reverse of the colony was the measurement recorded. These averages were computed from two measurements at right angles to each other of quintuplicate colonies. Since the test organisms produced circular giant colonies, the difference between the two measurements rarely exceeded one millimeter. The very rarely occurring replicate colonies of questionable diameter were subjected to the "Q" test, a simple screening test suggested by Dean and Dixon (**1951**) for use with small numbers of observations.

EXPERIMENTAL RESULTS AND DISCUSSION

A. Influence of Temperature.

Data contributed by many workers over the past years indicate that there probably is an optimum temperature for maximum

growth of each fungus; on either side of this temperature are temperatures inhibiting growth completely (Ames, **1915**; Miller and Golding, **1949**). A temperature allowing growth may not be favorable for the initiation of growth (Brooks and Cooley, **1917**). Moreover, differences in humidity or in concentration of the substrate may alter the temperature optimum for maximum growth (Groom and Panisset, **1933**; Bonner, **1948**). It has been observed that the effect on an organism of one temperature of incubation

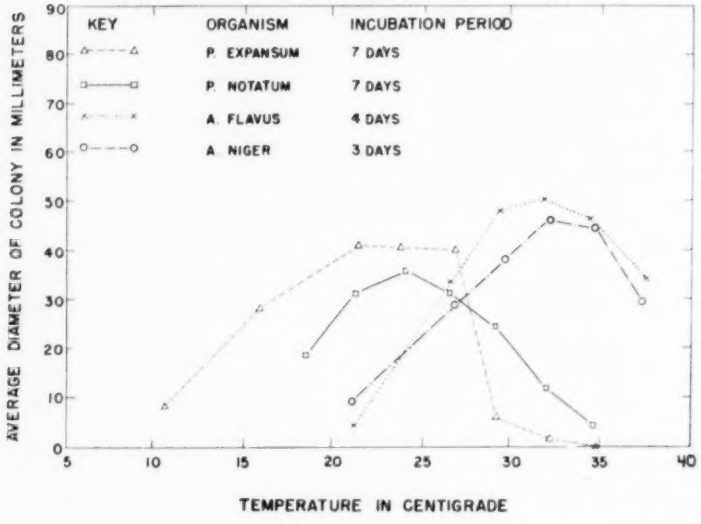


FIG. 1. Effect of variation in temperature on molds grown on malt agar.

may be discerned for a time after the organism is subcultured and incubated at another temperature (Fawcett, **1921**).

When four of the test organisms were grown over a wide range of temperatures, *A. niger*, *A. flavus*, and *P. notatum* yielded rather similar temperature-growth curves; *P. expansum*, however, exhibited some tolerance of temperatures lower than the optimum but a precipitous decrease in growth as the temperature rose above 26.7° C (FIG. 1). These growth curves are in accord with those obtained by Miller and Golding (**1949**) with the same organisms. All of these organisms have a favorable temperature range which

includes the optimum temperature and temperatures at which the diameter of the colony varies only a few mm from growth at the optimum temperature.

B. Influence of Concentration of the Medium.

In much of the work done on the influence of the concentration of the medium on fungus growth, dry weight data have been used as the measure of growth (von Mayenburg, **1901**; Weimer and Harter, **1921**; Wenck *et al.*, **1935**; Burkholder and McVeigh, **1940**, to mention a few). Where the diameter of the colony was used as the measure of growth, only the influence of small quantities of concentrating substances was studied (Golding, **1926**; Angell, **1929**; Galloway, **1935**).

TABLE I

EFFECT ON DIAMETER[1] OF MOLD COLONY (MM) OF ETHYLENE GLYCOL AND SODIUM CHLORIDE

Substance	Molal conc.	Organisms					
		A. niger	*A. flavus*	*G. candidum*	*P. notatum*	*P. roqueforti*	*P. expansum*
Molal concentrations of sodium chloride	0.00	38.3	52.9	80.6	32.6	63.6	38.0
	0.24	67.2	81.1	78.4	43.9	59.2	44.0
	0.47	57.2	72.7	56.1	44.5	47.3	40.2
	0.70	37.4	63.1	36.1	44.0	36.2	33.8
	0.94	28.5	54.8	18.0	41.8	25.8	28.8
	1.41	18.0	42.0	00.0	35.1	9.4	13.6
Molal concentrations of ethylene glycol	0.00	65.3[2]	52.7	80.2	31.0	53.7	35.1
	1.61	32.8	32.0	8.3	22.0	17.0	18.7
	3.32	4.5	6.2	0.0	1.8	5.3	1.0
	4.83	0.0	Germ.	0.0	0.0	Germ.	0.0
	6.44	0.0	0.0	0.0	0.0	0.0	0.0
	9.66	0.0	0.0	0.0	0.0	0.0	0.0

[1] Average of quintuplicate colonies.
[2] Incubated for 4 days.

The test organisms were grown on malt agar to which various quantities of concentrating substances were added. Glucose (Cerelose), sucrose (C and I), sodium chloride (Baker's Analytical C.P.), glycerol (Eastman Kodak), and ethylene glycol (Eastman Kodak) were used to concentrate the malt agar. FIGS. 2–4 inclusive and TABLE I illustrate the growth obtained when incuba-

tion was in the respective favorable temperature ranges of the organisms.

There was a stimulation of the rate of growth of all the molds by the lower concentrations of most of the substances. Ethylene glycol was the sole exception, exhibiting a marked toxicity which varied directly with its concentration. Although stimulation was most pronounced with the species of *Aspergillus*, the slope of the

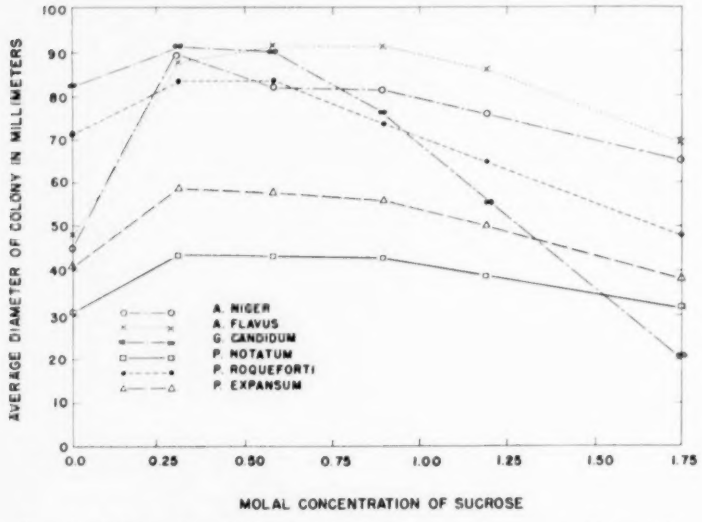


FIG. 2. Effect of variation in sucrose concentration on mold growth.

curve indicates that *P. notatum* was the most resistant to high osmotic concentrations. *G. candidum* showed the greatest inhibition by all the substances, and *P. roqueforti* was but slightly more resistant. The results of a study of probable causes of this stimulation will be reported at a later date.

C. Influence of Relative Humidity.

The importance of proper humidity conditions for the destructive growth of certain fungus plant pathogens such as the "powdery" and "sooty" mildews (Yarwood, **1935**; Fisher, **1939**), the rusts (Doran, **1922**; Stock, **1931**), the wood-destroying fungi

(Zeller, **1920**) and many others has often been noted. Likewise development of moldiness on butter (Macy, **1929**), on meat (Macara, **1943**) and on textiles (Galloway, **1935**) has been related to the occurrence of an atmospheric humidity suitable for mold growth. The effect of different relative humidities on the growth of various fungi has been studied chiefly on the basis of germination or non-germination of their spores or conidia. Rarely has the effect of different humidities on the diameter attained by the colony been recorded (Tomkins, **1929**).

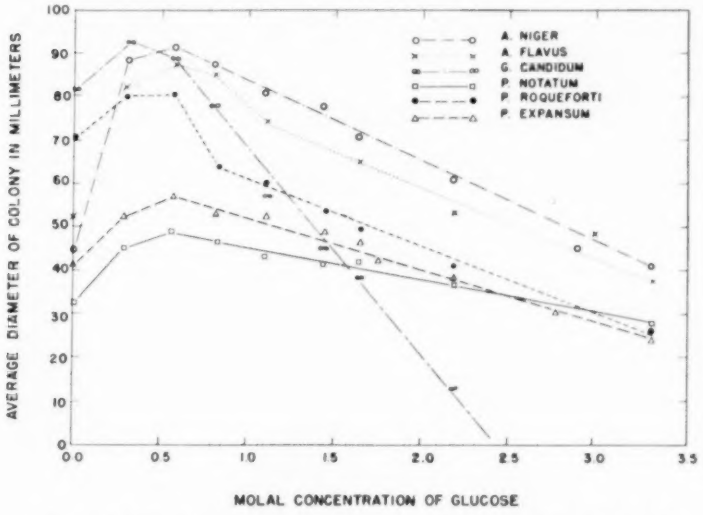


FIG. 3. Effect of variation in glucose concentration on mold growth.

P. roqueforti was used as the test organism in several experiments dealing with growth in atmospheres of various relative humidities. Desiccating jars were used as incubation chambers. In the first experiment there was no attempt to establish an equilibrium between the moisture in the substrate and the vapor pressure of the solution used in maintaining a constant relative humidity. TABLE II lists the humidity-maintaining substances along with the initial and the average relative humidities, as determined with a Serdex hygrometer, for the period of incubation. Also included in this table are the average diameters of colonies in dupli-

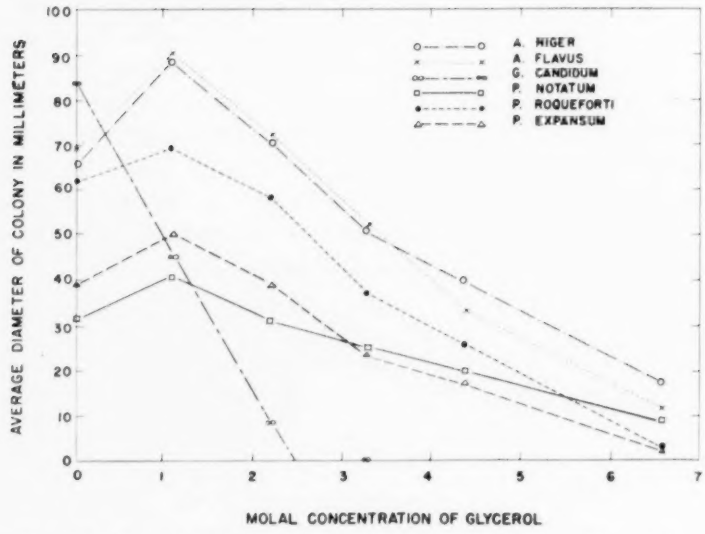


FIG. 4. Effect of variation in glycerol concentration on mold growth.

cate covered and uncovered plates. It is obvious that the relative humidity of the surrounding atmosphere had no influence on the diameter of colonies in the covered culture plates for the period of this experiment. FIG. 5 shows the morphological changes occurring at the higher relative humidity of closed plates. Similar results were obtained when uncovered plates were exposed to a relative humidity of 90 percent or higher. That the accumula-

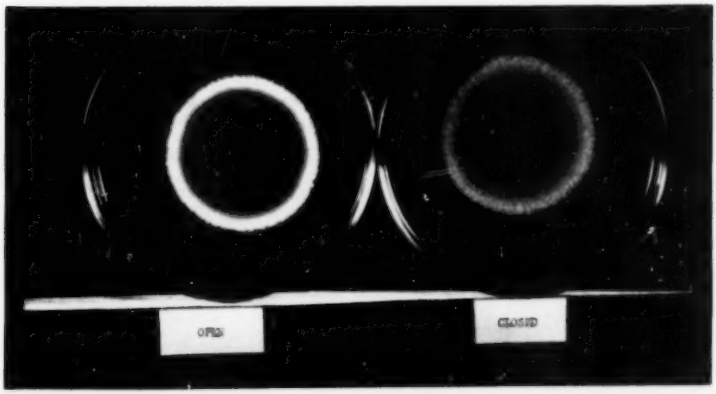
TABLE II

DIAMETER OF P. ROQUEFORTI[1] ON MALT AGAR AT VARIOUS RELATIVE HUMIDITIES

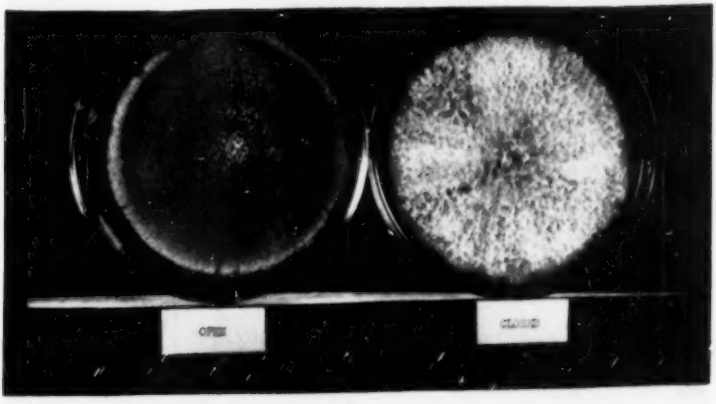
Humidity-maintaining substance	Average relative humidity	Average diameter in mm[2]	
		Covered plate	Coverless plate
H_2O	98.0	70.0	72.5
Sat. $(NH_4)_2SO_4$ Soln.	82.3	74.0	75.8
44% H_2SO_4	48.7	73.3	42.3
54% H_2SO_4	36.1	74.5	26.3
Anhydrous $CaCl_2$	13.2	73.8	19.0

[1] Incubation for 7 days at average temperature of 25.5° C.
[2] Average of duplicate colonies.

tion of carbon dioxide in closed plates had no part in this morphological change was shown by absorption of this gas with NaOH solution.



a. Penicillium roqueforti
(Incubation temperature 23.9°C)



b. Aspergillus flavus
(Incubation temperature 32.2°C)

FIG. 5. Mold cultures grown on malt agar in covered and uncovered petri plates at a relative humidity slightly over 80 percent.

In the second experiment, uncovered plates of media were not inoculated until an equilibrium between their moisture content and the vapor pressures of various aqueous sulfuric acid solutions had been established. The range of relative humidities was from 60.7 percent to approximately 100 percent. It was observed that the relative humidity most favorable for growth was between 80 and 85 percent. This result is in accord with data obtained by other

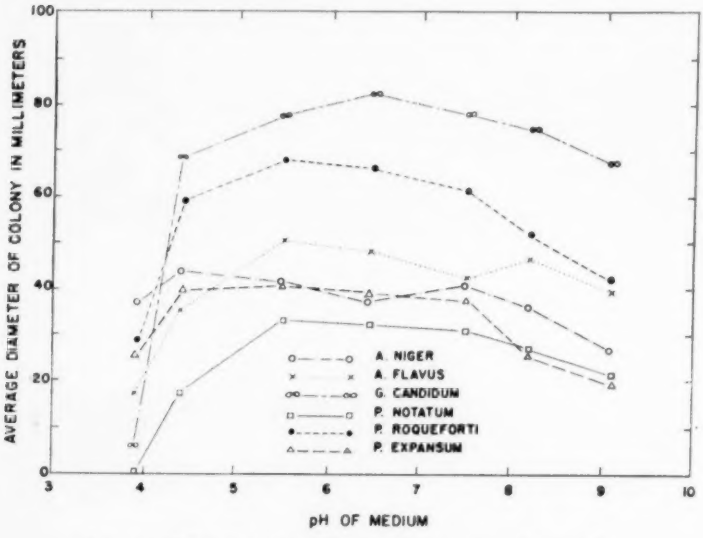


FIG. 6. Effect of initial pH of medium on mold growth.

workers with different species of Aspergilli and Penicillia (Lesage, **1895**; Tomkins, **1929**; Galloway, **1935**).

D. Influence of the Initial pH Value of the Substrate.

Investigators of the tolerance of fungi to varying hydrogen-ion concentrations agree that most of the fungi, and particularly those commonly called molds, have a broad region of pH insensitivity usually on the acid side of neutrality (Talice, **1930**).

In this experiment the reaction of sterilized malt agar (pH 5.5) was varied by the addition of sterile normal sodium hydroxide or normal hydrochloride acid. A Beckman glass electrode pH meter

was used to determine pH values. Quadruplicate plates of malt agar with initial pH levels of 3.9, 4.4, 5.5, 6.4, 7.5, 8.4, and 9.1 were inoculated with the six test organisms.

FIG. 6 presents the results as diameter in mm plotted against pH values. These curves with their broad plateaus of relative pH insensitivity show that the pH of the sterilized malt agar, 5.5, is optimum or very near optimum for all these organisms. *P. roqueforti* and *P. expansum* displayed the greatest and *G. candidum* the least sensitivity to alkaline substrates. *P. notatum* was the most sensitive and *A. niger* was the least sensitive to high hydrogen-ion concentrations.

The results of this experiment are in accord with the data reported by other investigators. Slight variations in the pH value of a medium between 5.5 and 7.5, occurring during its preparation, generally would not significantly influence the diameter attained by a colony.

E. Influence of Size of Spore Inoculum.

Brown (**1922**) studied the germination and growth of several common molds, including one species of both *Aspergillus* and *Penicillium*, under varied cultural conditions. He concluded that the density of the spore suspensions used as the source of inocula was most important when a weakly nutrient solution was used as the medium. On such a medium, growth diminished as the density of the spore suspension increased.

A study of the influence of the size of the spore inoculum upon the diameters of colonies of two of the test organisms was undertaken. For this experiment, *A. niger* was chosen as representative of molds which produce spores not readily wetted, and *G. candidum* was chosen because the preponderance of submerged growth in its colony suggested that its arthrospores were readily wetted. One ml aqueous spore suspensions were diluted with various amounts of sterile distilled water. Quintuplicate malt agar plates were centrally inoculated from each suspension in the usual manner, except that two of the plates were inoculated just before the agar solidified and then thoroughly shaken. Spore counts were made from the shaken duplicate plates after 24 to 36

hours of incubation at a temperature favorable for each organism.

TABLE III shows the two spore counts and the diameters of the three colonies for comparison. Under these experimental conditions, the diameter of the colony was independent of the size of the spore inoculum over a wide range.

TABLE III

COMPARISON OF SIZE OF SPORE INOCULUM TO DIAMETER OF COLONY ON MALT AGAR

Volume of spore suspension in ml.[1]	*G. candidum* (29.5° C)		*A. niger* (35° C)	
	Spore counts	Diameters in mm	Spore counts	Diameters in mm
25	65	58.5	19	46.0
	42	57.0	129	45.5
				45.5
50	62	58.0	83	45.0
	40	58.0	87	45.0
		58.0		46.0
75	34	58.0	15	45.0
	25	57.8	151	43.0
		59.8		43.0
100	44	58.0	49	43.5
	9	57.0	171	44.0
		58.8		44.5
150	46	57.5	22	43.0
	90	57.0	182	41.0
		58.0		42.5
Variation	9–90	57.0–59.8	15–182	41.0–46.0

[1] 1 ml aqueous suspension of spores diluted to various volumes with sterile distilled water.

F. Influence of Age of Inoculum Culture.

Methods for long-term preservation of culture or spore suspensions of fungi with a view to maintaining maximum viability have been described by many workers. It is apparent from these reports that the continued viability of many fungus spores is dependent on environmental conditions during dormancy or storage.

For the purpose of this study, it was desirable to know if any moderate variation in the age of the cultures used as sources of spore suspensions would influence the diameter attained by the

colony. Malt agar slant cultures of all the test organisms incubated at their respective favorable temperatures for 3, 11, 17, 22, and 30 days were used as source of inocula. Malt agar plates were inoculated in quadruplicate. The temperature and duration of incubation for each mold were the same used throughout this investigation. It was observed that there was no significant difference in the diameter attained by any of the molds that might be related to the age of the inoculum culture.

G. Influence of the Thickness of the Substrate.

Another step in the procedures used in this study, variations of which might conceivably have altered the diameter attained by the colony, was the use of 25 ml aliquots of media as the standard substrate for culturing the molds. The influence of variations in thickness of the substrate on diameter of the colony was shown to be nil by conducing a simple experiment.

TABLE IV

RELATION OF THICKNESS OF SUBSTRATE TO THE DIAMETER AND DRY WEIGHT OF THE COLONY ON MALT AGAR

°C	Organisms	Depth of medium			
		4 mm		1 mm	
		Av. diam. in mm	Av. dry wt. in mg	Av. diam. in mm	Av. dry wt. in mg.
35.0	*A. niger*[1]	45.9	19.7	46.3	11.0
32.2	*A. flavus*[1]	52.4	33.7	52.7	3.6
26.7	*P. notatum*[2]	27.8	14.3	28.5	12.4

[1] Average of quadruplicate colonies.
[2] Average of duplicate colonies.

Twenty-five ml aliquots of malt agar were placed in ordinary petri plates (diameter 90 mm) and in large petri plates (diameter 140 mm). The resultant depths of the media at the center of these plates were approximately 4 mm and 1 mm, respectively. Two plates of each size were used in culturing each of three test organisms. After the diameters of the colonies had been recorded, the mycelial pads were removed by melting the agar and,

following a thorough washing with hot distilled water, their dry weights were determined.

TABLE IV displays the average of two runs with *A. niger* and *A. flavus* and of one run with *P. notatum*. It is obvious that although the thickness of the substrate did not affect the diameter, the dry weight of the colony was materially altered. This was strikingly apparent in the case of *A. flavus*. The smaller amounts of spores and of aerial mycelium formed by the molds on the thin substrate no doubt account for the differences in weight. These results exemplify the chief criticism of using the diameter of the colony as a measure of growth: that a true record of the amount of growth actually occurring is not obtained.

H. Influence of the Restricted Atmosphere of the Petri Plate.

One factor, namely the atmosphere over the colony, has not been discussed. There are several facts which necessitate a brief consideration of this factor. First, it must be recognized that gaseous exchange betwen the atmosphere surrounding the culture plate and the atmosphere within the culture plate is restricted; the partial pressures of oxygen and carbon dioxide within the plates have an inverse relationship, the former lower and the latter higher, to their partial pressures normally in air. Aqueous vapor pressure is also considerably higher within the plates. Second is the observation that petri plates used in this study differed in vol-

TABLE V

RELATION OF LIMITED ATMOSPHERE OF PETRI PLATE TO THE DIAMETER OF THE COLONY

Organisms[1]	Days of incubation	Diameter in mm[2]	
		Covered petri plate	Coverless petri plate
A. niger	3	47.0	47.0
A. flavus	4	55.5	54.5
G. candidum	7	83.0	83.0
P. notatum	7	34.0	36.5
P. roqueforti	7	63.0	56.0
P. expansum	7	34.0	36.5

[1] Incubated at temperatures favorable for particular organisms and at approximately 80 percent relative humidity.
[2] Average of duplicate colonies.

ume. The importance of this factor can be quickly dismissed with the words of Stock (**1931**) concerning uredospores of rusts and spores of other fungi that, "Das Sauerstoffbedurfnis für Keimung und Keimschlauchwachstum ist ausser-ordentlich gering." Moreover, during 7 days incubation, the longest any experiment lasted, neither the lack of oxygen nor the accumulation of carbon dioxide proved to be a limiting influence on the diameter attained by the colony (TABLE V).

GENERAL DISCUSSION

Some environmental factors which might influence the colony diameter attained by a mold have been tested and evaluated with the view of determining the effect of variation of these factors. Replicate colonies generally showed very close agreement. The rarity of questionable diameters and the good correlation add support to the use of the diameter of a colony for (1) measuring the rate of growth of fungi, (2) comparing the influence of variously altered media on their growth, and (3) investigating the effects of other environmental factors.

The diameter of a colony lends itself satisfactorily to the measurement of the growth rate because there is no acceleration of the growth rate with time. Within a short time after germination, many molds attain a characteristic radial growth rate at optimum temperature. This rate is maintained for a period of time varying with the organism and the medium. Preliminary experiments had shown that this was true of several of the test organisms. *P. notatum* attained and maintained a growth rate of 6 mm (range 5–7 mm) per day for 12 days, while *P. roqueforti* maintained a rate of 12 mm (range 11–14 mm) for 7 days, when the plates were overgrown. This steady radial growth rate is due to the fact that only the apical cells of the filaments multiply. Therefore the colony's growth rate may be considered a composite of the rates of three factors related to morphology and physiology: (1) the rate of division of the apical cells, (2) the rate of branching (i.e., increase in number of apical cells), and (3) the rate of enlargement of cells (Rahn, **1948**). The fact that in some sectoring of fungus colonies the sector will disrupt the symmetrically radial

growth of the circular colony indicates that any one and probably all three of these factors may be gene-linked.

SUMMARY

The influence of temperature, concentration of the medium, relative humidity, reaction of the medium, size of spore inoculum, age of inoculum, thickness of the substrate, and atmosphere in the culture plate on the diameter attained by colonies of *Aspergillus niger*, *A. flavus*, *Penicillium notatum*, *P. expansum*, *P. roqueforti*, and *Geotrichum candidum* was studied. The results, in general, have been in accord with the data reported for other fungi and for different strains of the same species used in this work. Two of these factors, temperature and concentration of the medium, were observed to be important variables requiring careful control. The use of the diameter of the fungus colony as a measure of growth is considered sufficiently reliable for determining growth rates and for comparing the effect of environmental factors on a fungus cultured on the same medium. If an accurate knowledge of the the amount of growth is desirable, a cursory inspection of the giant colony will enable the researcher to judge whether or not this method of measuring growth introduced a gross error.

ACKNOWLEDGMENT

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FOUR YEARS OF MYCOLOGICAL WORK IN SOUTHERN SOUTH AMERICA

ROLF SINGER[1]

With headquarters at the Instituto Miguel Lillo in Tucumán, the author has endeavored to collect fungi in the tropical, subtropical, temperate, and subantarctic zones of South America, to obtain data on the types of species already described from southern South America, especially southern Brazil, Paraguay, Bolivia, Chile, Uruguay and Argentina, to build up a South American reference herbarium for Basidiomycetes, and to try to coordinate the results obtained in a taxonomic-floristic study of the Agaricales of Argentina and South Brazil, with some observations on the general character of the basidiomycete flora of southern South America and its possible relations to other floras.

Collecting trips were organized, according to a preconceived plan, to the historical collecting places of Spegazzini around Bueno Aires, to the Sierras of Cordoba, to the subtropical and alpine regions of the provinces of Jujuy, Salta, Tucumán, and Catamarca, to the Chaco, to the region of the Selva Misionera (Misiones and Rio Grande do Sul), to the tropical coastal forest of Brazil from Paraná to Rio de Janeiro, and, on the other hand, to the mountains of Patagonia and to Tierra del Fuego in the *Nothofagus* zone of the South. In many of these regions, classical localities (Bruch, Spegazzini, Rick) were revisited; in others the author found himself to be the first visitor looking for Basidiomycetes, or fungi in general. These collections, together with material purchased or obtained by exchange, form part of the Cryptogamic Herbarium of the Lillo Institute at Tucumán, and represent the largest collection of South American Basidiomycetes, and the largest fungus herbarium in South America. Syntypes of most

[1] The content of this article coincides in essence with an address given by the author for the Sociedad Botánica Argentina at the Spegazzini Institute Eva Perón, the 9th of September 1952.

of the new species are preserved at the Herbarium of the University of Michigan, Ann Arbor, Mich. A summary of the collections in Argentina was given in a paper in joint authorship with A. Digilio,[2] and a review of the South Brazilian work may be found in a paper now in print.[3]

It seemed, however, useful and necessary to supplement the purely taxonomic treatment by a summary of the more outstanding results obtained, such as may throw a light on the character of the basidiomycetous flora of the territory now explored, on the problems which have arisen in connection with this exploration, and to add some personal experiences which might be useful for those who will, in the future, add more information on the subject.

The first impressions of a mycologist in and near the large cities (Montevideo, Buenos Aires, Eva Perón, etc.) of the Rio de la Plata region are somewhat conflicting until it is understood that the character of horticulture and agriculture here is essentially that of the northern hemisphere. Since the host frequently determines the fungous flora, one is likely to find, side by side with native elements, species unwittingly introduced by man, either in the vegetative form (mycorrhiza with seedlings of pines in the pine plantations of Uruguay, in poplar plantations along the Rio Paraná, in willow rows, on *Eucalyptus*, etc.), or else as spores attached to various materials, seeds, etc., or together with cattle, horses, and sheep introduced from Europe (dung-inhabiting fungi). Thus, we find commonly species of the genus *Suillus* in plantations, even *Amanita phalloides* and *A. muscaria*, have been introduced, together with such edible species as *Tricholoma flavovirens, Leccinum nigrescens, Lactarius deliciosus*, all apparently from Europe, while *Gymnopilus spectabilis* ssp. *pampeanus* (Speg.) Sing. was apparently introduced from Australia. While it is comparatively easy to recognize the adventive character of the parasitic and mycorrhizal species, it becomes exceedingly difficult to establish the origin of the humus and dung-inhabiting forms. We found *Camarophyllus pratensis* in its European form in the

[2] Singer, R. y Antonio P. L. Digilio. Pródromo de la Flora Agaricina Argentina. Lilloa **25**: 5–462. 1953.

[3] R. Singer. Type studies on Basidiomycetes VI. Lilloa **26** (in press).

parks of Greater Buenos Aires and the assumption was tempting to consider this as an example of an introduced humicolous species. Yet, later on, a form indistinguishable from the European *C. pratensis* showed up in the subtropical-montane forest near the borderline between Tucumán and Catamarca, where the entire vegetation was native, and no agricultural activities were going on nearby. The genus *Camarophyllus* was also found to be native in Patagonia. A similar example is that of *Macrolepiota procera*. Some Argentine mycologists had believed that the common large *Macrolepiota* of the "pampa" of Buenos Aires province is merely of European origin, or at least occupies an enormous area including Europe. This is not so. The Argentine species is different from the European one, although very close, and is certainly native, since it is also found in remote mountain meadows in the interior and on grassy places in the Chaco. It is *Macrolepiota bonaerensis* (Speg.) Sing. Even more difficult is the determination of the origin of the supposedly subcosmopolitan dung-inhabiting fungi, such as various representatives of the genera *Coprinus, Stropharia, Deconica, Bolbitius, Conocybe, Pholiotella*, etc. These species may have been cosmopolitan from the start, yet their present wide distribution can also be explained by the fact that cattle and horses are now found everywhere, especially in Argentina, and these animals are all descendants of introduced European stock. The occurrence of some of these species on the dung of native animals such as guanaco, birds, etc. cannot be taken as proof that they also existed here in precolombian times, since they may have invaded this habitat after introduction with cows and horses, donkeys and sheep. All these facts—and in addition misdeterminations of native species by the determining mycologists (Spegazzini, Herter, Rick)—may account for the predominance of European and American species in the floristic lists of South Brazil, Uruguay, and Argentina, and in the only book for mushroom amateurs published in South America (Talice & Talice) as well as in the exsiccati distributed by Herter.

The picture changes completely as soon as one enters the native woods and forests. Already at the southernmost extension of the Selva Misionera (reaching from South Brazil to Misiones and

along the Rio Paraná south to Punta Lara), the percentage of fungi common to South America and the extratropical regions of the northern hemisphere becomes very small. *Agrocybe aegerita* is apparently one of them, *Schizophyllum commune* another. *Mycena pura* appears to be a third example, and so does *Galerina clavata* and *Hydropus fuliginarius*. A certain number of pantropical or "American" species (among the former, *Oudemansiella canarii, Panus crinitus, P. siparius, Coriolus pinsitus, Pycnoporus sanguineus, Whitfordia elegans, Coriolopsis occidentalis,* and many others; among the latter *Heimiomyces tenuipes, Laccaria tetraspora, L. ohiensis, Xerula chrysopepla*) are easily recognized by those who have some knowledge of tropical fungi and of the eastern North American flora, especially that of Florida. Yet, the number of endemic species is undoubtedly large, although, with our growing knowledge of the tropical fungi of other continents, the neotropical and endemic element is bound to become gradually less conspicuous in lists of native South American species. Examples of truly native species of the La Plata region seem to be *Alnicola devia, Crepidotus tigrensis, Paxillus argentinus, Friesula platensis,* but their precise area in South America cannot yet be circumscribed.

Traveling from the Rio de la Plata region northwest toward Bolivia, one passes the phytogeographic district of the Buenos Aires "pampa," which is a somewhat uniform stretch of almost treeless pastures characterized by rather abundant rainfall in spring and fall (April-June and August-September) and absence of snow, although freezing temperatures occur every year. Characteristic species inhabiting humus and dead herbaceous plants are *Amanita ameghinoi, Macrolepiota bonaerensis* and *M. kherandi, Volvariella cnemidophora, Marasmiellus caesioater, Marasmius pampicola, Clitocybe melliolens, Melanoleuca spegazzinii, Agaricus pampeanus, Bolbitius albiceps.* Common dung-inhabiting species are *Panaeolus subbalteatus, Anellaria sepulchralis, Agrocybe fimicola* (same as *Agrocybe coprophila* Sing. from Central Asia), which seem to be native, aside from many other species which may have been introduced but are now well established, such as *Stropharia semiglobata, Panaeolus phalaenarum,* etc.

Before the subtropical forest of the province of Tucumán is reached, one passes through the more or less xerophytic zone of the Chaco region (Chaco Santiagueño). This is a vegetation with scattered low trees, numerous cactaceous plants, and little or no herbaceous covering of the deep dry soil. There are entire seasons devoid of fungous growth because of absence of enough rainfall (except for occasional thunderstorms which provide enough moisture for a small number of saprophytic and parasitic Pyrenomycetes, Imperfecti, Gastromycetes, and half a dozen polypores, stereaceous fungi, and *Schizophyllum commune*, and a number of rusts and smuts, and hyphomycetous and phycomycetous soil fungi), but once in a while there is a period of prolonged (more than 3 days) rainfall and then an interesting fall flora of uniformly very small carpophores of agarics will appear, some of them with wide general distribution (*Conocybe lactea, Omphalina pyxidata, Crinipellis subtomentosa*), others, and this is the majorty, either endemic or at least restricted to the arid regions.

The forests of the so-called Selva Boliviano-Tucumana ascend in a characteristic zonation due to altitudinal climatic variations, the lowest belt being covered by a subxerophytic leaf-shedding forest with spiny undergrowth, forming some kind of transition between the Chaco formation and the subtropical rain-forest. Typical representatives of the basidiomycetous flora of this zone are *Tubaria omphaliopsis, Pluteus viscidulus, Leucoagaricus lilacinus, Lepista glabella*, various species of *Agaricus*, and *Marasmiellus, Fomitopsis sagracana* (Mont.) Sing. (*Fomes sagracana* Mont.), *Phellinus gilvus, Simblum sphaerocephalum. Heimiomyces tenuipes, Clitocybe scandens, Marasmius sanguirotalis, M. ruforotula, M. silvicola*, these and many others growing here are not limited to this zone, and even *Tubaria omphaliopsis* is often found in the drier zone of the montane *Schinus*-association. The subxerophytic zone reaches, in parts, deep into the subtropical zone along the ridges, whereas the true subtropical rain forest is most luxuriously developed in the narrow ravines ("quebradas") entering the Aconquija Mountains in a generally westerly direction. This forest is what may be called a "laurel" association (with *Phoebe porphyria* as the character tree). Here, we find a rich Basidiomycete-flora,

partly identical with that of the Selva Misionera to the far east, partly with species known to occur much farther north in the tropical forests of Orán, Bolivia and Perú, and the Upper Amazonas. Here again, the pantropical element is strongly represented (*Panus crinitus* and *P. siparius*, perhaps *Crepidotus citrinus* and *C. quitensis*, certainly *Diacanthodes philippinensis* and other polypores), while some species, according to our present knowledge, must be listed as endemics (*Phylloboletellus chloephorus*, *Marasmius bromeliaccarum*, *Phaeomyccna macrospora*, etc.). Species where the origin from farther north (up to Perú and Ecuador) can clearly be traced, are *Marasmius isabellinus*, *Crinipellis eggersii*, *Macrocystidia occidentalis*. "American" species are *Marasmius albogriseus*, *Crepidotus nephrodes* and *C. dorsalis*, again *Heimiomyces tennipes*, and various others. Representative examples of species occurring here as well as in the Selva Misionera (without having, as far as we know, a much further extended area) are: *Gyrodon rompelii*, *Marasmiellus byssisedus*, *Marasmius hymeniicephalus*, *M. leoninus*, *M. magnificus*, *M. silvicola*, *Neopaxillus echinospermus*, *Calocybe cyanea*, *Armillariella puiggarii*, *Hydropus fuliginarius*, *Resupinatus subrhacodium*, *Collybia collybioides*, *Cantharellula coprophila*, *Rhodocybe himantiigena*, *Heimiomyces pruinatipes*, *Marasmius puttemansii*, *M. echinatus*, *Crepidotus acanthosyrinus*, *Hohenbuchelia paraguayensis*, *Lentinus puiggarii*, *Panus microspermus*, *Pleurotus rickii*, *Pluteus viscidulus*, *Favolaschia pygmaea*. Since the Selva Boliviano-Tucumana has been studied for basidiomycetous fungi for four years by the author and less intensely by others, it may be of some value to add a list of species which do occur in the Selva Misionera east to Porto Alegre, but have never been encountered in northwestern Argentina: *Clitocybe aberrantissima*, *Trogia cantharelloides*, *Callistosporium luteofuscum*, *Melanoleuca balansae*, *Chaetocalathus liliputianus*, *Crinipellis macrospaerigera*, *C. misionensis*, *Filoboletus gracilis*, *Hydropus riograndensis*, *Pluteus riograndensis*, *Macrocystidia carneipes*, *Marasmiellus microscopicus*, *M. fascicularis*, *M. niveus*, *M. subfumosus*, *M. subpumilus*, *M. tropicalis*, *M. vinosus*, *M. viridiluteus*, *Marasmius iguazuensis*, *M. ilicis-macrodonti*, *M. neosessilis*, *M. pallipes*, *M.*

trichorhizus, Xerula pilosa, Pholiota apiahyna, Hebeloma austro-americanum, Inocybe hyperythra, Galerina arenaria, G. minor, Crepidotus croceotinctus, C. palmarum, C. uber, Rhodocybe gilvoides, Leucopaxillus brasiliensis, L. gracillimus, Ripartitella brasiliensis, Lepiotella brunnea, Leucoagaricus rubrofibrillosus, Agrocybe retigera, Conocybe macrorhina, C. missionum, Xerocomus brasiliensis, Russula puiggarii, Russula riograndensis, Lactarius braunii, L. russula, Favolaschia rubra, F. puiggarii, F. saccharifera, Arrhenia pezizoides, A. cyphelloides, Brauniella albida, and, of course, all those species growing on *Sphagnum* (collections from Taimbesinho, Rio Grande do Sul) and *Araucaria* (such as *Tricholomopsis araucariae*). A list of species occurring in northwestern Argentina and north to Perú but not observed in southern Brazil would serve no purpose since in spite of the many collections from the latter region, there are still many species waiting to be discovered and added to the list published by us.[2]

Returning to the zonation of the Selva Boliviano-Tucumana, we ascend now from the true subtropical rain forest into the subtropical-montane belt, also called fog-forest, and dominated by myrtaceous trees. This zone is—at least mycologically—poorly and indistinctly separated from the lower zone, and there are only a few species worth mentioning as specific for this formation, usually occurring on *Eugenia* sp. or on *Chusquea*, which forms the underbrush: *Mycena micromphale, Rhodophyllus argentinus, Lentinus sayanus.*

The uppermost zone is markedly cooler than the others, and consists, as far as trees are concerned, of three associations, the coniferous woods (the lowest, in general, formed by *Podocarpus parlatorei*), the alder woods (the most extensive, formed by *Alnus jorullensis* var. *spachii*) and the "genoa" woods (generally the highest, bordering the subalpine meadows, formed by a rosaceous tree, *Polylepis australis*). This zone of montane woods with, (except for the numerous epiphytes and lack of a permanent snow cover in winter) almost boreal aspect, had hitherto never been visited by a mycologist or anybody collecting fungi. While the lower zones of the Selva are—with one exception, which we shall mention later—devoid of non-endotrophic mycorrhiza, the *Alnus*

woods show an abrupt predominance of Agaricales of the mycorrhizal type as generally common in regions where representatives of the Fagales and Salicales, *Pinus* and related conifers, *Tilia* and *Fraxinus* are common. While in the lower zones the average size of the fruiting bodies of the Agaricales is small, we find here some of the familiar "northern" type of fleshy fungi, one species of *Lactarius*, one *Gyrodon* (*G. montanus*, strangely a closest relative of the European *G. lividus*), three Russulas (quite different from the type of primitive tropical species represented in Venezuela and Brazil), at least two Cortinarii and one *Inocybe*. Naturally, we also find the genus almost regularly associated with *Alnus* formations and forming mycorrhiza with this tree, the genus *Alnicola* (with two species). It was *Alnicola diplocystis* which gave us first the necessary evidence of the presence of ectotrophic mycorrhiza in the Alnetum since the rootlets of *Alnus* from Catamarca were surrounded by the hyphae of this species (see Schweiz. Zeitschr. Pilzk. **28**: 185. 1950 and Sydowia **4**: 153. 1950). Only very few species of the lower belts are occasionally found in the *Alnus* woods, and even then they are rare, and evidently intruders (*Marasmius berteroi*, *Heimiomyces tenuipes*, the latter, as may be expected, being the most versatile of all species occurring under all possible conditions from extreme dryness in the subxerophytic forest to the extreme moisture of the South Brazilian jungle, and from tropical hot climate (Trinidad) to montane temperature zones, attacking any woody substratum living or dead from coniferous wood to monocots). On *Podocarpus*, we have found interesting isolated specimens of a northern genus, *Xeromphalina*, a species closely related to *X. campanella*, always occurring on conifers in the northern hemisphere, here replaced by *X. helbergeri*. On *Alnus*, we have a similar example in *Flammulina velutipes*, a winter-fruiting species of the northern hemisphere, never before observed in South America. Some of the non-mycorrhizal species occurring in the Alnetum also grow in pure stands of *Polylepis*, e.g., *Clitopilus argentinus*, *Clitocybe scandens*, and *Mycena pura*. *Polylepis* and *Podocarpus* do not form ectotrophic mycorrhiza, as shown by careful observations during three consecutive seasons. The same seems to be true for

Araucaria in Brazil and Patagonia, and for *Pilgerodendron*, *Fitzroya* and *Libocedrus* in Patagonia.

However, complete absence of mycorrhiza of the ectotrophic kind cannot be claimed for the subtropical forest. It is true that the genera of Agaricales usually forming the ectotrophic mycorrhiza in pine woods and in Fagales woods are absent; but there is one notable exception: *Gyrodon rompelii*. It is known that *Gyrodon lividus* and *montanus* form mycorrhiza with *Alnus*, and *G. merulioides* and perhaps *G. proximus* with *Fraxinus*. The tree-relation of *G. rompelii* remained a puzzle. Finally, it could be shown, by direct observation of tree roots and by a careful analysis of the tree flora around several populations of this species, that a new family of mycorrhiza-bearing angiosperms has to be added to the list: The Sapindaceae. The mycorrhiza host seems to be almost invariably *Allophylus edulis*.

Above the rain forest and the montane woods, we enter the subalpine meadows, and subsequently the alpine zone of the Pre-Andes and Andes. In certain regions such as the valley of Tafi (Tucumán) and Suncho (Catamarca), we encounter, aside from species more specific for this region (*Melanoleuca tucumanensis*, *Conocybe reticulatorugosa* and many other Conocybes, *Omphalina rustica*), various species already familiar from the "pampa" of the plains such as *Marasmius pampicola*, *Bolbitius albiceps*, *Macrolepiota bonaerensis*, *Agrocybe fimicola*, and *Anellaria semiovata*. In the alpine zone, from which no specimens had been known until now, a number of very interesting species were recovered, notably *Rhodocybe claudopus* in *Pycnophyllum-convexum* (Caryophyllaceae)-tufts, *Melanoleuca sparrei* and *Collybia sleumeri*, and finally, the species reaching higher than any other basidiomycete and at present holding the world altitude record: *Marasmius albogriseus*, collected by B. Sparre at 4250 meters in the Calchaquies Range of the Preandine Sierras.[4] Naturally, in high-alpine regions with less precipitation, as in the alpine desert known here as Puna region, fungal growth becomes as rare and sporadic as phanerogamic vegetation, and only rarely an occasional

[4] Basidiomycete mycelium, apparently of *Hohenbuehelia pycnophylli* was collected by Sleumer in the province of Salta at 4500 m., February, 1953.

gastromycete is encountered. Only two Agaricales are known from such places, and as the time of fungus fructification here is not predictable, a methodical exploration of these regions is practically impossible. It is the same situation that makes us dependent on occasional collections by local inhabitants or travelers, here in the mountain deserts as well as in the great southern desert of western Patagonia. Nevertheless, even these random collections yield extraordinarily interesting material such as the type collection of *Galeropsis allosperma* Sing. (*Galera paradoxa* Speg.), the first and only species of *Galeropsis* without germ-pore, and therefore very significant in the interpretation of the phylogeny of Secotiaceae; for all Bolbitiaceae have spores with germ-pore, and the existence of a gastromycetoid form with spores without germ-pore, which in a group with thick complex spore walls is a relatively primitive character, can justly be used as an argument in favor of the derivation of the Bolbitiaceae from the genus *Galeropsis* rather than vice versa.

When judging lists of collections of Basidiomycetes from the various provinces of Argentina and Chile, from Paraguay and Brazil, northern Uruguay, and the eastern mountain slope of Bolivia, it should not be forgotten that some of the localities in seemingly very dry regions are actually marginal forests along smaller and larger rivers. It is these types of vegetation that form the "bridge" between the "Selva Misionera" and the "Selva Boliviano-Tucumana." Thus, a species common to both Selvas may also be found in marginal forests along rivers crossing the Chaco between them. A characteristic example is the interesting endemic genus of South America, *Neopaxillus*, with its only species *N. echinosperma*. *Salix humboldtina*, usually found in this type of vegetation, is often accompanied by its most conspicuous mycorrhizal partner *Hebeloma austroamericanum* and a lignicolous species, *Pholiota pseudofascicularis* (*Flammula alnicola* var. *salicicola*).

In order to coordinate the study of Rick's Brazilian types with collecting trips to the type localities and adjacent regions of the Selva Misionera where it is most luxuriant, two trips were organized, one to the Argentine northeast spur between Paraguay

and South Brazil, known as the territory of Misiones, the other, upon invitation by Dr. B. Rambo, S.J., to the Brazilian state Rio Grande do Sul. It is extremely difficult to find out about the collecting seasons in such regions, but acting on the best information available, we chose late spring and early summer in both cases. It seems, however, that basidiomycetous carpophores, even fleshy fungi, appear theoretically every month of the year, although, in spite of the frequent rainfalls, the summer crop of carpophores may be rather reduced because of possible short dry spells while such dry spells would not affect the necessary moisture minimum in winter or later fall; nevertheless, during winter, there is again the danger of cold spells (nights below freezing do occur) which stops the formation of carpophores for considerable time in some seasons; this is understandable since the majority of the Basidiomycetes, as we shall soon see, seem to be of tropical origin and have a rather high optimum and minimum temperature for mycelium growth as well as for carpophore production. More prolonged and careful observations would be necessary in order to determine whether or not there is a division into summer-fruiting and winter-fruiting Agaricales as could be observed in northern Florida, USA (see Farlowia **2**: 97. 1945). Unfortunately, the Misiones trip was somewhat hampered by a dry spell and so was the Rio Grande trip; yet by visiting the moistest spots, swamps as well as the surroundings of waterfalls, a considerable amount of interesting material was gathered. Our impression is, however, that in spite of Spegazzini's short trips, our own two collecting expeditions, and the long period of years devoted to the collecting of fungi near Porto Alegre by Rick, the mycoflora of this best known section of Brazil is still apt to yield a large number of thus far unrecorded or misdetermined and too poorly described material. Rick did not believe in the value of dried material, so that many of his species are not represented in his herbarium, or else only by specimens hardly referable to the original concept of his species. The mycological flora of the Selva Misionera is incredibly rich. According to careful observations of Padre Rambo, the "campo" or grassland vegetation, characteristic for northern Uruguay, is gradually retreating, and the "selva" vegetation seems

to be gaining ground at the present time. This makes one think that the elements of the subtropical forest here consist mainly of species of tropical origin migrating from the north into Rio Grande do Sul, and carrying with them those species of fungi which are at the same time adapted to the hosts migrating south, and able to stand the climatic change (colder winters, occasional freezing temperatures although in most cases the microclimate in which the mycelium develops hardly ever reaches temperatures even near 0° C).

A few scattered collections by the author in the coastal tropical forest to the North of Rio Grande do Sul, especially in Paranaguá (State of Paraná, Brazil) and Angra dos Reis (State of Rio de Janeiro, Brazil), an analysis of the still existing material collected by various individuals and described by Rick, Spegazzini and Hennings from the states of São Paulo and Santa Catharina, Brazil, and finally the type material of some of Montagne's Brazilian species as well as those collected in the first half of the last century by Gardner and Spruce in Brazil—all these data taken together give a rather poor picture of the actual percentages of the mycoflora of both subtropical and tropical Brazil; in other words, it is impossible at present to check on Rambo's theory by an analysis of the existing mycological data. All we can do at present is indicate a number of species common to the tropical forests of Santa Catharina, lower Paraná and São Paulo, Rio de Janeiro, Minas Geraes, etc. on one hand and Rio Grande do Sul or Misiones on the other. No attempt is made to be complete. We merely give a few examples of such species on the basis of personal acquaintance with the material: *Collybia collybioides, Cantharellula coprophila, Marasmius magnificus, Marasmiellus eburneus* (Theissen) Sing. (*Marasmius eburneus* Theissen), *Leucoagaricus rubrofibrillosus, Russula puiggarii.* There is, on the other hand, no doubt, but that numerous species of the tropical Brazilian forest even south of the Amazonas, do not reach the South Brazilian subtropical forest, just as might be expected. Thus, there is no indication of *Marasmius ferrugineus* (unless it be identical with *M. spegazzinii*), *Marasmius albocapitatus,* and some Marasmielli, Collybiae, and Rhodophylli collected by me be-

tween Paraná and Rio de Janeiro, either among Rick's specimens at Porto Alegre or material collected by me in Rio Grande do Sul. Yet, there is naturally no proof that they do not occur at all. Recent papers of Dennis treating his agaric collections in Venezuela and Trinidad are a valuable addition to our knowledge of the fungous flora of northern South America, and one will encounter a large number of the species mentioned here, although some of Dennis' interpretations are not fully identical with those accepted in our papers. For example, what we call *Marasmius haematocephalus* is *Marasmius tageticolor* in Dennis, what we call *M. tageticolor* is an acystidiate species (the type) not mentioned in Dennis, and what Dennis calls *Marasmius haematocephalus* is a species unknown to me. What Dennis describes as *Marasmius aciculaeformis* is not that species, since the latter (type) has smaller spores while Dennis' species is probably *M. pallipes* Speg. His *Collybia cycadicola* is evidently *Lactocollybia angiospermarum*, and his *Micromphale rheicolor* is evidently our *Heimiomyces tenuipes*. His *Dictyoploca plectophylla* is either incorrectly determined (spores of type nonamyloid) or else there is a mistake about the amyloidity of the walls in the description, a circumstance which may also have led him to underestimate the value of this character in certain groups of Agaricales. *Marasmius leoninus* in the sense of Dennis is different from the type as well as from material of that species occurring in Brazil and analyzed by the author. *M. rhabarberinus* Berk., type, also differs from Dennis' concept of *M. leoninus* and from ours by having definitely free and more distant lamellae. These few remarks on an otherwise valuable and very instructive paper are necessary in order to show that in many cases even the comparison of the results of modern authors for comparative phytogeographical studies is rather risky, and may lead to wrong conclusions.

A particularly interesting phase of the mycological studies in Brazil was the study of fungi growing on *Araucaria*, and those found in the *Sphagnum* bogs of the higher altitudes in the Serra north of Rio Grande do Sul toward Santa Catharina. Dennis and I each found one representative of the genus *Tricholomopsis* in South America at approximately the same time, mine growing

on *Araucaria* in Brazil and being extremely close to the type species of the genus. Until then, the occurrence of this genus in thermophilous populations was only suggested by the existence of several Floridian representatives of the genus. The upper margin of the plateaus at about 1000 meters elevation showed very little Agaricales growth in otherwise seemingly very suitable conditions (mixed woods of *Araucaria, Podocarpus, Ilex* and other evergreens mixed in, with numerous tree ferns and mossy ground cover) in November. But the reason may have been a seasonal appearance of the Agaricales in this almost temperate climate with no previous experience as to the periodicity of fruiting in this particular region, which had never been visited previously by anyone collecting fungi. On the other hand, in the nearby *Sphagnum* bogs, a comparatively rich flora of sphagnophilous Galerinas, *Psilocybe uda, Collybia dryophila* var. *oedematopus* etc. was observed by us at the very same time, and it is likely that the flora of the *Araucaria* association in this altitude and latitude is comparatively poor. The character of the sphagnophilous fungous flora is decidedly boreal, especially as far as the acystidiate *Psilocybe uda* is concerned while, among the Galerinas, we found some evidently endemic species, a feature to be expected in a locality so isolated and peculiar in its flora components.

No mention is made of the pantropical or neotropical element and the cosmopolitan species. It would be easy to enumerate quite a few species found here as well as in northern South America, Cuba and Florida and in some cases also in tropical Africa and Asia. Particularly impressive by their numbers in every association studied and by their extremely common occurrence are *Schizophyllum commune, Pleurotus ostreatus* (cosmopolitan species) and *Filoboletus gracilis* (from Rio Grande do Sul north to Florida, and also outside America) and other species with similar distribution (*Collybia plectophylla, Dictyopanus pusillus, Coriolus occidentalis, Mycobonia flavida, Agrocybe aegerita, Panus rudis, P. crinitus and P. siparius, Clitopilus incrustatus, Rhodophyllus squamifolius, Oudemansiella canarii* and *steffenii, Polyporus tricholoma, Pseudofavolus cucullatus*, also many species of *Coriolus* and *Fomitopsis, Phellinus* and *Whitfordia*), and such American spe-

cies as the ubiquitous *Heimiomyces tenuipes, Crepidotus nephrodes* and *C. croceotinctus, Gymnopilus peliolepis* and *G. chrysopellus.*

Following the Andes and preandine sierras south, we find a wide xerophytic gap between the southernmost extension of the Selva Boliviano-Tucumana and the north tip of the *Nothofagus* woods of the South. This gap was studied mycologically by Spegazzini who received some scattered collections from the Cuyo region (including the important provinces of San Juán and Mendoza) and a rather large and complete collection made by Bruch in the province of Córdoba. Aside from a short trip to the latter province, we have not visited the treeless grasslands and deserts, the partly wooded mountain ravines and the parks and plantations of that region. Even a survey of the type studies on Spegazzini's material and our own experience does not warrant any attempt to enounce a theory as to the origin and character of this mycoflora. Its gastromycetous component is apparently rich and interesting; the agarics consist of some endemic as well as intruding elements from adjacent regions and of some species of wide distribution such as various polypores and *Bolbitius fragilis,* and the only bolete of the region, *Phacogyroporus tropicus* ("hongo de Córdoba").

South of the nearly treeless gap, we find the Andes again covered by woods; in the northern part of West Patagonia intermixed with such more northern genera as *Chusquea* and with a rich myrtaceous flora, but basically, here as well as south to Tierra del Fuego, consisting of various species of *Nothofagus.* It is well known that the genus *Nothofagus* is one of the best examples of an area disjunction between Australia and Western South America. While the majority of the remaining phanerogamic vegetation does not show the same disjunction, it is important to note that the majority of the fungi are intimately linked with the most important host plants, the Nothofagi, and, consequently, show less kinship with the mycoflora of the central and northern part of the Andes and preandine Sierras than the phanerogamic flora. Before a full appreciation of this interesting aspect of the flora of Patagonia can be given, it would be useful to study what seems

to be the center of distribution of the basidiomycetous flora of this region, the woods of Valdivia in Chile. These woods have not been studied systematically thus far; only occasional collections from local collectors are available at Santiago, Chile, and in the Spegazzini herbarium. Earlier collections were sent to Montagne by Gay, and were published in Gay's famous *Historia Natural de Chile*. But this enumeration likewise contains only very few species so that it is necessary to depend on my own collections from east of the Chilean border and reaching to the borderline between Argentina and Chile. While a rich mycoflora is maintained in a coastal strip of land south of Concepción, Chile, to the south tip of the continent and then bending towards the east and reaching the Isla de los Estados (Staten Island), the mycoflora as well as the diversity of the *Nothofagus* species and associations formed by them becomes less striking while the sum of yearly rainfall decreases from west to east, and the margin of the woods is rarely very far from the Chilean frontier. East of the margin of the woods is the wide Patagonian desert, and only in the southern part of the Argentine sector of Tierra del Fuego do the woods reach the Atlantic coast. Rains, and with them Basidiomycetes, appear both in spring and—more abundantly—in fall, further south mainly in early fall.

Since *Nothofagus* belongs to the Fagales, there can be confidence as to which of the numerous large fleshy Basidiomycetes form ectotrophic mycorrhiza with these trees. Such mycorrhizal relations could indeed be demonstrated in the same way as they were first demonstrated in the Alneta of the Selva Boliviano-Tucumana. The latter, being the nearest wooded area with Fagales, was naturally suspected of having served as a bridge for the southward migration of species from the north tip of the area of *Alnus jorullensis* (Mexico) to the *Nothofagus* area. However, this idea turned out to be wrong: it is not borne out by the facts. The flora of the Alneta contains indeed very few species which also occur in the *Nothofagus* woods of the south according to detailed studies made by the author in numerous trips to the Alneta as well as in two extended expeditions to the *Nothofagus* area (Northwestern Patagonia: Neuquén and Rio Negro; Tierra del

Fuego: Rio Grande to Lago Fagnano). Leaving out of consideration such apparent cosmopolitan species as *Armillariella mellea* in the wider sense, *Clitopilus scpticoides*, *Schizophyllum commune*, *Mycena pura*, *Bjerkandera adusta*, *Laccaria echinosperma*, etc., the similarity between the Nothofageta and Alneta of South America boils down to the fact that they have altogether two species in common. One is *Crepidotus brunswickianus*, and the other is *Naucoria polytropa*. In the case of the latter, there even seem to be certain differences between the characters of the respective collections. More species may be added in years to come, but our present knowledge is certainly good enough to predict that the list of species common to the Nothofagetum and the Alnetum will never be large. There is likewise no resemblance between the Basidiomycetes observed on or under *Salix humboldtiana* further northeast and the same class of fungi observed in the *Nothofagus* area. *Laccaria tetraspora* (of wide distribution) and *Collybia platensis* seem to be the only species common to the two phytogeographic districts. On the other hand there is a definite relationship between the fungi of the *Nothofagus* area in South America and the fungi of New Zealand and Australia. Forms closely related to *Thaxterogaster* (South America) and *Descolea* (South America) have recently been observed by Heim in New Zealand; both Amanitae occurring in the *Nothofagus* area have their nearest relatives among Australian species (one seems to be identical with an Australian *Amanita*). We may add that the ascomycetous genus *Cyttaria* has actually the same distribution as *Nothofagus* itself. A better knowledge of the basidiomycetous flora of New Zealand will undoubtedly increase the number of examples. Aside from that, we have in Northern Patagonia and even more, the further south we go, an increasing number of species with a bipolar disjunction of area, species which are definitely identical with boreal species of the northern hemisphere and may as such be compared with the current example in phanerogamic botany: *Primula farinosa*, occurring both in the Patagonian Andes and in the mountains of the northern hemisphere. Such examples of fungi are *Omphalina griseopallida*, *Galerina vittaeformis*, *Inocybe cutheles*, *Clitocybe suaveolens*, *Melanoleuca excissa* and

M. evenosa, Hygrophoropsis aurantiaca, Hohenbuchelia petaloides, and in other cases we may indicate pairs of corresponding, extremely closely related species of the same stirps, one occurring in the boreal regions of the northern hemisphere, the other in the *Nothofagus* area: *Camarophyllus lacmus* as reported from Iceland, and *C. adonis* from Tierra del Fuego, *Pluteus atromarginatus* (Sing.) Kuehner and *P. spegazzinianus* from Tierra del Fuego, *Clitocybe leptoloma* from the United States and Canada, and *C. subleptoloma* from Tierra del Fuego, *Melanoleuca cognata* and *M. longispora, Phacomarasmius distans* and *P. chiliotrichi, Bondarzewia berkeleyi* and a new species of *Bondarzewia* on *Nothofagus dombeyi* in the Nahuel Huapi region, and finally *Leucopaxillus rhodoleucus* and *L. patagonicus.* The explanation of this disjunction is perhaps one of the most challenging problems of historical plant geography.

The mountain meadows within the *Nothofagus* area show a rich fungous flora containing *Armillariella pumila, Omphalina defibulata, Camarophyllus tehuelches, Agaricus pampeanus, Galerina clavata, Marasmiellus fibula;* the numerous *Sphagnum* bogs contain *Galerina riparia, Naematoloma myosotis;* the alpine zone has not been touched so far. Saprophytic species, characteristic of the woods are, among others: *Fistulina antarctica,* three species of *Lepista,* several species of *Clitocybe,* two species of the smooth-spored group of lignicolous species included in *Fayodia* by me, *Pluteus jaffuelii* (Speg.) Sing. (*Mycena jaffuelii* Speg.), one *Lepiota* of the *acutesquamosa* group, one new *Kuehneromyces, Crepidotus brunswickianus* and *C. nephrodes, Coprinus atramentarius* vel aff. and *C. comatus* v. aff., *Tubaria gregoriana,* many species of *Melanoleuca, Panellus longinquus,* species of *Clavariella, Glocosoma vitellinum.* Among the mycorrhiza-forming Agaricales: Several species of *Porpoloma, Russula, Cortinarius, Inocybe, Tricholoma, Amanita,* one *Hebeloma,* one *Descolea.* Among the Secotiaceae, we noticed *Thaxterogaster maghellanicus* (very common), and *T. violaceus* (always sessile), both formerly collected by R. Thaxter near Punta Arenas (Chile); among the Discomycetes we observed numerous Helotiaceae and Morchellae and *Gyromitra,* several species (at least three) of *Cyttaria.* The

number of polypores is not so large as in the tropical and subtropical regions, which is characteristic for the boreal-subarctic and for the subantarctic regions in general. There is, however, *Crinipellis ursina* (Speg.) Sing. and *Favolaschia antarctica* (Speg.) Sing. apparently growing on *Nothofagus;* there is furthermore *Marasmiellus couleu* growing on *Chusquea couleu*, and *Campanella dendrophora* Sing. & Dig. spec. nov. ined. on the same host. All of these, including the *Chusquea*, are species with northern relations, their respective genera having their most conspicuous development in the warmer regions of the continent. But these are exceptions. In general, the predominantly "tropical" genera are absent (*Nothopanus, Trogia, Pleurocollybia, Dictyopanus, Phaeomycena, Lactocollybia, Cymatella, Physalacria, Chaetocalathus, Hymenogloea, Heimiomyces, Filoboletus, Chlorophyllum, Leucocoprinus, Cystoagaricus, Melanophyllum, Smithiomyces, Ripartitella, Copelandia, Melanotus, Pleuroflammula, Pyrrhoglossum, Ncopaxillus*) and even such genera as *Panus, Lentinus, Marasmius* were rarely encountered. More interesting, because, without reference to relative thermophily of the respective organisms, is the complete absence of representatives of the genus *Hygrophorus*, of *Alnicola*, of all Boletaceae (with the exception of *Boletus loyo*), all Strobilomycetaceae and Gomphidiaceae, the entire genus *Lactarius*, the genus *Hypsizygus* and *Pleurocybella, Tricholomopsis, Lyophyllum, Macrolepiota, Limacella*, all Cantharellaceae, etc., at least among the reported collections on the Chilean side, and on the entire Argentine side visited by me. Among the few genera that seem to have a particularly rich representation, so far as species as well as individuals are concerned, we name *Naucoria, Cortinarius* (and *Descolea*), and *Paxillus*. The large number of species of *Cortinarius* and related genera does not surprise those who understand the importance of the discovery of *Thaxterogaster*, the secotiaceous genus which seems to have been the starting point of this group of agarics (see Mycologia **43**: 215–228. 1951) but the variety in the Paxillaceae in southern South America in general, and in Patagonia in particular, is amazing. While it was thought that the presence of clamp connections is a family character in the Paxillaceae, we now find one species, *P. defibu-*

latus, without clamp connections, and we find two species of *Paxillus* with distinct veil (*Paxillus argentinus* from the Buenos Aires region, and *Paxillus boletinoides* from Patagonia-Tierra del Fuego).

Leaving the native woods and entering the extensive experimental plantations of conifers on Isla Victoria, Lago Nahuel Huapi, Neuquén, we find *Suillus luteus* conquering the entire pine area of the island as well as in nearby gardens and parks, regardless of the origin of the particular species. This, compared with the somewhat less exclusive but definitely dominant mycorrhiza of *Suillus granulatus* in the Rio de La Plata region, creates a new problem for forest mycologists interested in forestation. How can the mycologist help the forester in these problems? It seems obvious that the importance of the mycorrhizal partner at least in the nursery stage of the forestation project can hardly be exaggerated. It appears, then, that it is a further fact that the same pines, under different climatic and soil conditions, form mycorrhiza with different mycorrhizal partners. In order to establish the seedlings, it is consequently desirable to control the mycorrhizal association rather than to leave it to chance as is done at present. Another case which, in my opinion, shows that the exploration of the mycological flora of Southern South America is not merely a scientific necessity, is the discovery—not by a phytopathologist—of the above mentioned species of *Bondarzewia*,[5] a species highly damaging to the most valuable lumber resource of Patagonia. Its correct classification gives the forest pathologist, together with the observation, a hint where to look for analogous tree diseases (*Bondarzewia montana* on *Abies nordmaniana* in the Caucasus, *B. berkeleyi* on oak in North America). Another example is the observation of *Phacogyroporus* in the area of intensive citrus cul-

[5] **Bondarzewia perniciosa** spec. nov. Pileo intense aurantiaco (pl. 4 A 12 vel "Pujab" Maerz & Paul), concentrice depresso, tomentoso. 140–160 mm. lato. Tubis dilute aurantiacis, breviusculis, irregularibus, decurrentibus. Stipitibus in truncum basalem dense concrescentibus carpophorum complexum multipileatum usque ad 1 m. latum formatibus. Carne tenacissima, aurantiaco-albida vel alba, admodum piperata. Sporis eis *B. montanea* (Quél.) Sing. similimis, amyloideo ornamentatis, subglobosis. Ad truncos vivos *Nothofagi dombeyi* arboresque necans. Typus Quetrihué, Neuquén, Argentina, Maio 1952, R. Singer M *608*.

ture in Argentina (Tucumán) where it causes the phytopathologist to watch for a dreaded disease reported in the Rio de Janeiro region by Brazilian phytopathologists, and connected with this bolete whose cryptas create the conditions under which the pathogenic organisms attack the roots. A similar threat is the presence in Patagonia, the Buenos Aires region, Brazil and Chile of either *Armillariella mellea* or a very closely related parasitic species, *A. puiggarii*.

Some of the species reported by us are edible and either now used by mushroom gatherers (the *Suillus luteus* of Isla Victoria is even the object of a concession given to a company in Bariloche for its exploitation in the National Park of Nahuel Huapi), or else of potential importance in the future. Most of the species of *Agaricus* growing in the pampa and mountain meadows belong in this category as only one plant producing canned white mushrooms—in mushroom houses in Martinez near Buenos Aires—operates at present. *Phaeogyroporus tropicus* is collected and dried for export into other provinces in the Sierras of Córdoba, and is used instead of imported *Boletus edulis* in oil (from Italy). Other edible species are: *Macrolepiota bonaerensis, Agrocybe aegerita, Armillariella mellea* and *puiggarii, Lepista diemii* (from Patagonia), *Boletus loyo* (from Patagonia, especially from Chile), *Mycenastrum corium* (here often called *M. chilense*) and other "puffballs," *Lactiporus sulphureus, Morchella*. We have established the edibility of *Marasmius pampicola, Pluteus spegazzinianus, Cantharellula tarnensis, Laccaria tetraspora*. Among the poisonous native fungi, we mention *Chlorophyllum molybdites, Amanita ameghinoi*, a little known species of *Volvariella*, and several species of *Inocybe*, probably also other species of *Amanita* found in the north and south of Argentina and in Chile, and a certain undetermined number of Lepiotas (in the widest sense).

The scientific significance of the material collected and described and the observations made in connection with this cannot be measured by the—perhaps impressive—number of new forms discovered. The only classification at hand applicable to the non-European Agaricales flora is that of the author (Lilloa 22, 1951), and we have always considered this system of classification

as being on trial by nature itself when it was attempted to insert the multitude of unknown species and of species formerly incompletely known into this new, or rather, partly new system. The fact that four years of most intensive research work in South America has unearthed only four new genera in the Agaricales and one in the Gastromycetes—and this without a large number of adjustments of the limits of the existing genera[6]—is taken as a favorable result as far as this system of classification is concerned. On the other hand, it was possible to add certain forms of taxonomic interest to the system, such as *Phylloboletellus*, the long-expected lamellate genus in the Strobilomycetaceae, *Gloiocephala* Mass. as re-collected and recharacterized (the *Marasmius* with smooth hymenophore), *Thaxterogaster* and *Brauniella*, the two new links between Gastromycetes and Agaricales from Tierra del Fuego and South Brazil respectively, the gemporeless spe-

[6] These adjustments are so few that they can be enumerated here. The group of *Clitocybula*, inserted as a subgenus in *Fayodia* in my classification, contains Patagonian species with completely different habit. I do not hesitate to admit that this proves me wrong and Josserand and others who have insisted on the separation of this subgenus from the type subgenus (*Fayodia*, formerly *Eu-Fayodia*) were apparently right. It is proposed to consider *Clitocybula* a genus and transfer it to the *Leucopaxilleae*, in the neighborhood of *Cantharellula*. The genus *Asterotus*, thus far monomorphic, had also to be emended in order to accommodate a closely related but gelatinous species from Argentina. This suggests that *Asterotus* would be better placed in the neighborhood of *Resupinatus* than in its present position. A close observation of the forms of *Poromycena* in comparison with the section *Purae* of *Mycena* leads me to the conclusion that *Poromycena* is better considered a section or subsection of *Mycena*. A clearer understanding of Heim's proposed unit *Phacomycena* leads me to the conclusion that this latter is intermediate between *Lactocollybia* and *Callistosporium*, and both *Phacomycena* and *Lactocollybia* should be transferred to the neighborhood of *Callistosporium*. *Paxillus* must now be characterized as having clamp connections except for one species, and in *Panellus*, we must now remove the words "cystidia none," saying that cystidia do occur on the sides of the lamellae in some species of *Panellus*. By the discovery of more species in *Heimiomyces*, it appears now that this latter genus is closer to *Xeromphalina* than had been anticipated, even though the two were placed side by side in my classification. However, I do not think that, at the present time, there is any need of contemplating the generic identity of the two genera. But the number of new species inserted in *Armillariella* and *Marasmiellus* may make it possible to consider a division of each of these genera into two independent ones.

cies of *Galeropsis*, *G. allosperma* Sing., the species transitory between the section *Tubariopsis* and *Galerina* discovered in Brazil. Aside from that, certain genera which—although well known as such—consisted until now only of one species, were found to contain several species, and the delimitation from the neighboring genera was made more secure. A further interesting aspect was the fact that those groups which were thought to be characterized not merely by a set of correlated morphological characters but by their ability to form ectotropic mycorrhiza with certain forest trees, were consistently absent where the mycorrhiza partners were absent, a confirmation of their absolute dependency on these hosts that could have been obtained only in difficult and not always fully convincing experimental work with hundreds of species involved. This aspect of our results has been dealt with in a special communication given to the XIIth International Botanical Congress and now in print.

Some of our observations seem to indicate that the subtropical rain forest is not—as it may seem—an absolutely ideal habitat for the normal type of fleshy fungi. Such a habitat is much rather the humid part of the "pampa," or the northwest Patagonian woods. In the Selva Boliviano-Tucumana, there are two factors detrimental to the normal development of fleshy Basidiomycetes: (1) stronger competition by other soil organisms, Actinomycetes, Hyphomycetes, bacteria, with a tendency toward faster destruction by decay of the larger carpophores; (2) stronger and more rapid changes in humidity of the humus and the air than in both truly tropical and temperate forests. The result is a tendency toward smaller carpophores, or more leathery or gelatinous carpophores, or else a tendency toward potential abbreviation of the life cycle achieved by sclerotized gonotoconts, and elimination of basidiospore formation and dispersion, or else by elimination of the reduction division (and hence abolishment of hymenophores). An interesting example of a species that forms basidiospores only in a nearly primordial stage and later abandons basidium formation in favor of large vesiculose terminal cells of the subhymenium which eventually sclerotize, is *Marasmius spinosissimus* Sing. It is the first case of a single organism being

a true agaric at first, and forming something closely approaching a *Rhacophyllus* form toward the end of its development. It may therefore serve as proof of the basidiomycetous character of *Rhacophyllus*. This is no new statement. Indeed, it is currently assumed that *Rhacophyllus* is a form of an agaric, but this statement was based on nothing more than an analogy in the cytology of the bulbils and the basidium, and the similarity of the shape of the carpophores. An example of imperfect agaric fructification could be given by describing the formation of both arthrosporial and basidiosporial fructifications in species of an *Armillariella, A. ditopa,* also from near Tucumán. The same mycelium may form both imperfect and perfect carpophores, the former clavarioid, the latter clitocyboid; but one is separated and independent from the other.

A modern critical review of South American species also throws a different light on the relative prevalence of certain genera in the tropics and subtropics. Using statistics derived primarily from Saccardo, Sylloge, one would assume that the richest genera in species inhabiting the tropics are *Pleurotus, Panus,* and *Lentinus, Collybia, Marasmius, Omphalia,* and *Lepiota.* This is only true as far as the last genus is concerned, and to a certain degree for *Marasmius* if *Marasmiellus* is included, also to a certain extent for *Collybia. Pleurotus, Panus,* and *Lentinus* are poorer in species, due to the fact that an enormous amount of later synonyms have been described, and this again is due to the fact that many of the species have been simultaneously described in all three of these genera, and many in some additional genera. This, in its turn, was unavoidable because of the lack of a criterium as to what characters should be attributed to these genera.

During our past studies on the material collected, certain groups were somewhat neglected, not because of lack of material or observation but rather because of the policy that guided the entire plan of work in South America, which consisted in the belief that specialists of certain genera would be better equipped to master the quantities of material and notes than nonspecialists. This caused me a certain delay in the determination of some Hygrophoraceae, *Mycena,* Agaricaceae and Coprinaceae, *Cortinarius*

and *Rhodophyllus*. It is, however, not really of any great influence in the interpretation of our material as a whole, since, in the tropical-subtropical belt all these groups, but *Lepiota* in the wider sense, and *Agaricus* in the narrow sense (Agaricaceae), and in the south all but *Cortinarius*, are of no more than average importance as constituents of the basidiomycetous flora. The vast and seemingly indomitable genus *Lepiota* needs a special and careful study, careful especially because of the fact that many species of this genus have been described in other genera not even related to it, by both Rick and Spegazzini. The study of *Agaricus* will not merely bring some order into the multitude of South American forms of a very important genus; it will, beyond that, influence the development of *Agaricus* studies in the northern hemisphere, since even preliminary type studies show that some northern species occur here, and some of these have probably been described in Argentina or Brazil (Rick frequently described them in *Pholiotella*) before they were described in Europe or North America. *Agaricus* has also practical importance. For regions like Tucumán or Rio de Janeiro, it will be necessary to select a local or pantropical species, or anyhow, a species with a higher optimum growth temperature than observed in *Agaricus bisporus* (Lange) Sing., the commercially grown species. Furthermore, as I have pointed out, there are numerous indications which suggest that *Agaricus* plays some rôle in the fungus-ant symbiosis so characteristic for wide stretches of South America. A study similar to that made on the relationship of termites and *Termitomyces* in the palaeotropics should result in South America as soon as more is known about the genus *Agaricus*. There should be no difficulty in growing the fungus frequently collected by entomologists (who like to call their symbiotic ants "agriculturists") to fructification. Even if the perfect form of the species involved were not *Agaricus*, the latter must have at least an epibiotic relationship to the fungus-ant complex.

Geographically speaking, there is no major white spot left in the fungus map of Southern South America. Nevertheless, there are minor spots of interest. A more careful collecting program with the aim of establishing the relation between the mycoflora of

the Selva Boliviano-Tucumana and the Upper Amazonas should plan visits to Orán, the Cochabamba forests in Bolivia, and the eastern slope of the Andes in Perú. Collecting in the territory of Formosa and all the way down along the Paraná will undoubtedly open up certain unknown subfloras in and around the wetter part of the Chaco, and clear up its mycological relation with the Selva Misionera. A trip to Valdivia will round out our knowledge of the northern portion of the Patagonian *Nothofagus* area, and collecting in the subalpine and alpine regions of the Southern Cordillera might mean the opening up of an untouched field. This can easily be done by studying the mycoflora of relatively accessible places like the western part of the mountains around Lago Argentino. It is also hoped that hosts like *Fitzroya* (at the Laguna Frias or in Chile), and the study of llama and guanaco and *Hippocamelus* dung in remote places of South America might throw more light on the question as to whether any of the common coprophilous species are indigenous in this continent. As for the understanding of historical mycogeography in the great lines, the exploration of the continent should now be directed to its immense and practically untouched *terra incognita*, the Amazonas basin and the Upper Orinoco, the northwestern mountain range in Ecuador and Colombia with special attention to be paid to the boreal elements (if such exist) along the upper limits of vegetation and in the oak forests of Colombia. South American mycologists will also show great interest in the further exploration of the basidiomycetous flora of the *Nothofagus* area of New Zealand.

Even though our knowledge of the continent as a whole is sketchy, and perhaps not more than a quarter or one half of the species of southern South America has been mapped, it is now possible to say that a start has been made, that further work has been outlined and made easier by the existing type studies and the prodromus of an Argentine agaric flora and a survey of the South Brazilian basidiomycetous flora. This was possible thanks to the farsighted and inspired organization of biological research initiated and directed by the Director of the Instituto M. Lillo, Dr. Horacio R. Descole and, also, by the faithful and disinterested cooperation of the—unfortunately so few—botanists scattered all over southern

South America, especially B. Rambo, J. C. Lindquist, J. E. Wright, Espinosa, Diem, Cárdenas, and my colleagues at Tucumán. An acknowledgment is also due to the financial support given to the mycological work carried out by the Universidad Nacional de Tucumán, the Fundación Miguel Lillo, and the Guggenheim Memorial Foundation.

NEW SPECIES OF GALERINA FROM NORTH AMERICA [1, 2]

ALEXANDER H. SMITH

In the following account twenty-eight heretofore undescribed species of *Galerina* are treated. These were collected in the Great Lakes region and along the Pacific Coast during the period from 1935 to 1952. As a rule, species of *Galerina* are not a conspicuous element in our agaric flora, but during the warm dry season of 1952 they were found in abundance in Mount Rainier National Park. During the fall of 1952 no appreciable amount of rain fell in the Park from around the tenth of September until the end of October, and the forest floor, ordinarily covered during this period with fruiting bodies of many species in various genera of the fleshy fungi, was barren. Fungi were found only at high elevations and in local pockets where there was moisture and protection from the sun. One notable area of this sort was at Green Lake in the Carbon River district of the Park. The north-facing slopes of the Tatoosh Range furnished many favorable spots, such as that around Snow Lake.

Collecting was carried on in these areas as well as at higher elevations until long after the usual time for the fall snows and the advent of winter. Thus the 1952 season in the Park was truly unusual, and served well to illustrate the need for collecting in an area under all types of weather conditions before drawing many conclusions about either the diversity of a fungous flora or the fruiting pattern of the species comprising it. During the season of 1948, one of the wettest years in the history of the Park, the same areas produced practically nothing in the way of fungi during the summer and were covered by snow early in September.

[1] Papers from the University of Michigan Herbarium and the Department of Botany, University of Michigan, Ann Arbor, Mich.

[2] Published out of order as excess pagination at cost of University of Michigan Herbarium.

Kühner (1) monographed *Galerina* for France as part of a treatment of the Friesian genus *Galera*. In this work he emphasized spore structure as one of the important diagnostic characters. As he defined the genus, the smooth suprahilar depression or "plage" of the spore was the central character, and this has been followed by Singer (3). It is also followed in the present paper. Both earlier authors included entirely smooth-spored species in the genus, as I have done here, and a few in which the suprahilar area as well as the rest of the spore surface is roughened. However, the latter species are regarded as peripheral. Assignment to *Galerina*, in such instances, is of course made on the aggregate of all the other characters.

In his work Kühner treated eighteen species. I have accounted for practically all of these in North America; the twenty-eight additional species treated in this paper are only the more outstanding discoveries that we have made. In fact, when the study is complete, I predict that there will be around sixty species from North America. Thus *Galerina* is not a small genus, as some have thought.

Smith and Singer are at present engaged in a monographic study of the genus, and for this reason illustrations of carpophores, drawings of microscopic characters, and discussions of relationships and speciation are not presented here. These can all be treated more effectively in a work dealing with all known species.

The type collections are deposited in the University of Michigan Herbarium. In regard to species collected in our national parks, if the collections were large enough to be divided, a part has been deposited in the Mount Rainier National Park Herbarium. The color terms in quotation marks are taken from Ridgway.[3] The observations on the spores were made from dried specimens revived in 2.5 percent KOH, and viewed with a 1.3 NA oil-immersion objective and a 15 × eyepiece. This is important to anyone seeking to compare spores with the descriptions published here because the spore markings are exceedingly minute in some species.

[3] Ridgway, R. Color standards and color nomenclature. Washington, D. C. 1912.

The collecting expeditions on which these fungi were obtained were made possible largely by grants from the Faculty Research Fund of the University of Michigan during the years from 1935 to 1952, and in part by the generosity of Mr. Wm. B. Gruber of Portland, Oregon. The University of Michigan Herbarium also financed some of the field work during this period, and facilities for the collecting in northern Michigan were furnished by the University of Michigan Biological Station located on Douglas Lake.

I am particularly indebted to the National Park Service for its cooperation in furnishing facilities, equipment, and other aids upon a number of occasions. I wish, again, to express my personal appreciation to Superintendent Preston P. Macy and his staff for courtesies extended to me on my visits to the Olympic National Park in 1939 and 1941; to Mr. John C. Preston, then superintendent of Mount Rainier National Park, for courtesies extended to my party at Mount Rainier during the season of 1948. Superintendent Preston P. Macy, now of Mount Rainier National Park, and his staff were of great assistance during the dry season of 1952. It was through the guidance of members of the naturalist staff that the best localities were found and visited during 1952.

1. **Galerina atkinsoniana** sp. nov.

Pileus 3–5(–7) mm latus, 3–5 mm altus, conicus, fulvus vel pallide fulvus; lamellae distantes, subventricosae, late adnatae; stipes 2–3 cm longus, c. 1 mm crassus, aequalis, subpubescens; sporae 11–14 × 6.5–8 μ; pleurocystidia 38–65 × 10–15(–16) μ; pilocystidia pleurocystidiis similia. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Upper Falls, Tahquamenon Falls State Park, Mich., Aug. 23, 1951, Smith n. *38184*.

Pileus 3–5(–7) mm broad at base and 3–5 mm high, obtuse becoming campanulate to convex, glabrous (or pubescent under a lens), moist, hygrophanous, "russet" to "tawny," fading to "warm buff" (fulvous to tawny, fading to yellowish), striate to apex when moist, flesh very thin, watery-fragile, tawny to ochraceous, odor and taste not distinctive; lamellae broadly adnate, broad (1.5 mm), distant, subventricose, tawny at maturity, pale ochraceous when young, edges white-fimbriate and slightly eroded; stipe 2–3 cm long, about 1 mm thick, equal, fragile, lower part concolorous with or darker brown than pileus, paler above, or at first con-

colorous throughout, densely pruinose from projecting caulocystidia, no veil present on buttons.

Spores 11–14 × 6.5–8 μ, ovate-apiculate in face view, slightly inequilateral in side view, rugulose to minutely warty as revived in KOH, some showing 1–3 globules in the interior, a very minute apical pore present as seen under oil; basidia 2-spored, 27–34 × 7–8 μ, projecting slightly when sporulating; pleurocystidia scattered, fusoid-ventricose, 38–65 × 10–15(–16) μ, thin-walled and hyaline in KOH; cheilocystidia abundant to scattered, 28–46 × (8–)9–14(–17) μ, narrowly to broadly fusoid-ventricose, thin-walled, hyaline in KOH; gill trama subparallel to interwoven; cells short and relatively broad, pale ochraceous from incrusting pigment when revived in KOH; pileus trama homogeneous, pale ochraceous to tawny in KOH, pigment incrusted and more conspicuous (darker) toward cap surface, no appreciable pellicle differentiated, pilocystidia present and similar to the pleurocystidia, clamp connections present; caulocystidia abundant, hyaline, thin-walled, 60–120 × 9–18(–20) μ, fusoid-ventricose.

Habit, habitat, and distribution: Scattered among mosses on the ground or on old logs, apparently northern and not common. It is to be expected during the summer and early fall.

Observations: This species is characterized by the large pilocystidia resembling the pleurocystidia. Both 2-spored and 4-spored pilei have been found. The pruinosity on the stipe is caused by numerous caulocystidia. The collections referred here were first placed in *Galerula muricellospora* Atkinson, until a study of Atkinson's type revealed that it contained no carpophores with pilocystidia.

2. **Galerina camarinoides** sp. nov.

Pileus 4–7 mm latus, obtuse conicus, demum late conicus, pallide argillaceus; lamellae distantes, late adnatae, latae; stipes 10–15 mm longus, c. 1 mm crassus, pruinosus, dilute ochraceus; sporae 9–11 × 5–6 μ, pallide ochraceae; cheilocystidia 18–36 × (3–)4–8(–9) μ, ventricosa et capitata vel cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope East Fork, Salmon River, Mt. Hood, Ore., 3,800 ft. alt., Smith n. *19374*.

Pileus 4–7 mm broad, obtusely conic, the margin appressed against the stipe when young, becoming broadly conic, near cinnamon buff on disc, margin paler and near pinkish buff, translucent-striate, hygrophanous, becoming slightly paler and opaque when

faded, glabrous at all times; flesh very thin, concolorous with surface, odor and taste not distinctive; lamellae distant, 8–10 reach the stipe, 1 tier of lamellulae or none at all, broadly adnate, broad, pale ochraceous tawny, edges even; stipe 10–15 mm long, ± 1 mm thick, equal, fragile, pale honey color, coarsely pruinose clear to apex.

Spores 9–11 × 5–6 μ, subelliptic in side view, elliptic in face view, ochraceous tawny or somewhat darker in KOH, very minutely punctate-roughened with a smooth suprahilar depression, apical pore present; basidia two- and four-spored, 26–30 × 8–9 μ, hyaline in KOH; pleurocystidia none; cheilocystidia 18–36 × (3–) 4–8(–9) μ, cylindric-capitate to ventricose at base and capitate at apex, neck often very narrow (± 3 μ), hyaline and thin-walled in KOH; gill trama interwoven, pale yellow in KOH; pileus trama near surface tawny in KOH from heavy incrustations of pigment, clamp connections frequent; caulocystidia 30–62 × (4–)6–12 μ, variable in shape but the general pattern cylindric to subventricose with capitate apices.

Habit, habitat and distribution: Gregarious on moss over rock, East fork of Salmon River, Mt. Hood, Ore., 3800 ft. elev., Sm-*19374*.

Observations: The cheilocystidia are definitely the *G. sideroides* type but the spores, under a good oil immersion lens, are seen to be typical of *Galerina*. No veil was noted in my collection, but in Hesler *17642* a faint band of fibrils is visible on the stipe indicating veil remnants. This fungus cannot be *G. camerina* (Fr.) Kühner because the stipe is not "*umbrino*" as Fries described it.

3. **Galerina carbonicola** sp. nov.

Pileus 8–17 mm latus, conico-campanulatus, glaber, "russet" vel "tawny" (non latericius); lamellae adnatae, confertae, angustae; stipes 4–6(–8) cm longus, 1.5–2.5 mm crassus; deorsum subbadius; sporae 8–10.5 × 4.5–5.5 μ; cheilocystidia 35–45 × 9–12 μ, fusoidi-ventricosa; carbonicola. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Whitmore Lake, Mich., Sept. 28, 1933, Smith n. *33-1032*.

Pileus 8–17 mm broad, conic-campanulate to convex and with a small obtuse papilla, margin at first often curved in against gills, conic-campanulate, glabrous, hygrophanous, when moist "russet" or "tawny," when faded "ochraceous buff" (yellowish), striate nearly to apex, opaque when faded; flesh thin, fragile, watery-cinnamon brown when moist, yellowish when faded, odor and taste

not distinctive; lamellae adnate to slightly adnexed, close, rather narrow (± 2.5 mm) but finally somewhat ventricose near stipe, concolorous with pileus both when young and mature, edge minutely fimbriate; stipe 4–6(–8) cm long, 1.5–2.5 mm diam., concolorous with pileus or a little paler above and darker below, minutely pruinose above, scantily appressed-fibrillose below, some appearing longitudinally striate from appressed fibrils, base usually slightly bulbous to evenly enlarged.

Spores 8–10.5 × 4.5–5.5 μ, narrowly subinequilateral in side view, narrowly ovate in face view, russet in KOH at maturity, the outer layer present as irregular warts or deeply wrinkled, suprahilar depression smooth, apical pore present but indistinct; basidia four-spored, 24–28 × 7–8 μ, hyaline in KOH, projecting half their length when sporulating; pleurocystidia, none seen; cheilocystidia abundant, 35–45 × 9–12 μ, fusoid-ventricose, the necks often flexuous, the apices subacute to obtuse; gill trama more or less interwoven, rusty brown to yellow revived in KOH; pileus trama homogeneous, hyphae loosely interwoven and rusty brown from incrusting pigment, becoming pallid ochraceous on standing in KOH, no pellicle differentiated but hyphae of pellicular region radial and many narrower than those of tramal body; clamp connections present.

Habit, habitat and distribution: Under brushes on burned area, Mud Lake Bog, Whitmore Lake, Mich., Sept. 28, 1933, Sm-*33–1032*.

Observations: The russet to tawny color of pileus, gills and stipe, the rudimentary veil, the narrowly ovate spores in face view and the fusoid-ventricose cheilocystidia are distinctive, along with the carbonicolous habitat. Pleurocystidia similar to the cheilocystidia are to be found around portions of the hymenium damaged, possibly, by insects.

4. **Galerina cortinarioides** sp. nov.

Pileus 1–2 cm latus, late conicus, dilute fulvus, striatus; lamellae confertae, adnatae, latae, subfulvae; stipes 3–4 cm longus, 2–3.5 mm crassus, fragilis, deorsum demum subbadius, albofibrillosus, sursum albo-zonatus; sporae 8–11 × 6–7 μ; cheilocystidia 32–46(–55) × 6–10(–12) μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Sept. 18, 1952, Smith n. *40019*.

Pileus 1–2 cm broad, obtuse with a straight margin, expanding to broadly conic or margin spreading and disc a flattened umbo, surface glabrous except for a few marginal fibrils, hygrophanous,

"ochraceous tawny" or darker moist and then translucent-striate, ± cinnamon buff faded; flesh thickish but soft and fragile, watery ochraceous, taste slightly rancid (not farinaceous), odor none; lamellae broad, close, adnate, ventricose, pale to dark ochraceous tawny, edges crenulate; stipe 3–4 cm long, 2–3.5 mm thick (thickish as in *Cortinarius*), very fragile, pallid-brownish above, darker below and base bister in age, surface copiously decorated with remnants of a white veil but apical zone soon evanescent.

Spores 8–11 × 6–7 μ, ovate in face view, inequilateral in side view, ochraceous tawny, outer wall wrinkled, fitting like a sack over the spore and with loose ragged boundaries at hilar end, suprahilar depression smooth and marked by a ragged line or blisters of loosened exosporium; basidia four-spored, 23–30 × 8–9 μ; pleurocystidia none; cheilocystidia abundant, 32–46(–55) × 6–10 (–12) μ, fusoid-ventricose with long neck and obtuse apices, hyaline, or some with yellow content as revived in KOH, thin-walled; gill trama regular to subinterwoven, ochraceous in KOH from incrusting pigment; pileus trama with a thin pellicle of narrow (3–5 μ) nongelatinous hyphae with pale ochraceous-tawny incrusting pigment, tramal body of broader hyphae and also with incrusted pigment; clamp connections present.

Habit, habitat and distribution: Gregarious on soil in trail to Green Lake, Mt. Rainier National Park, Wash., Sept. 18, 1952, Sm-*40019*. Additional collections are Sm-*40097* and *40860* also from the Park.

Observation: In aspect this fungus resembles the small Cortinarii around *C. acutus* but the spores place it in *Galerina*.

5. **Galerina cuspidata** sp. nov.

Pileus 10–15 mm latus, 10–12 mm altus, cuspidatus, viscidus, dilute-cinnamomeus; lamellae confertae, latae, ventricosae, adnatae, pallide argillaceae; stipes 2–3 cm longus, 2–2.5 mm crassus, sparsim fibrillosus; sporae 8–9 × 4.5–5 μ (10–12 × 5–5.5 μ), leves; cheilocystidia 18–32 × 4–6.5 μ, anguste ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Upper French Creek, Medicine Bow Mts., Wyo., Aug. 14, 1950, Nancy Jane Smith (A.H.S. n. *35716*).

Pileus 10–15 mm broad at base, 10–12 mm high, sharply conic to cuspidate, becoming conic campanulate, margin straight at first, surface viscid, glabrous or with flecks of fibrils from veil along the margin, "Sayal brown" (dull cinnamon) moist, hygrophanous, fading to yellowish or finally pallid, moist margin only faintly striatulate; flesh thin, pliant, concolorous with surface, odor and

taste none; lamellae close to crowded, broad and ventricose, adnate to adnexed but ascending, "pinkish buff" young, gradually becoming "cinnamon," edges even; stipe 2–3 cm long, 2–2.5 mm thick at apex, equal, pallid above, honey color below, base darker in age, surface thinly fibrillose from remains of pallid veil, apex naked to minutely pruinose.

Spore 8–9 × 4.5–5 μ (11–12 × 5–5.5 μ), smooth, no apical pore visible (under oil), ellipsoid, in side view the ventral line straighter than the dorsal; basidia four-spored (some 2-spored?); pleurocystidia none seen; cheilocystidia 18–32 × 4–6.5 μ, narrowly fusoid-ventricose to narrowly ventricose-subcapitate, often variously contorted; gill trama parallel, the subhymenium gelatinous; pileus trama cinnamon in KOH except for the gelatinous pellicle of narrow hyaline hyphae (3–5 μ); clamp connections present.

Habit, habitat and distribution: Gregarious on moss in a springy area, North Fork, French Creek, Medicine Bow, Mts., Wyo., Aug. 14, 1950, coll. by Nancy Jane Smith (A.H.S.-*35716*).

Observations: This species is related to *Galerina sideroides* but is easily distinguished in the field by the cuspidate pileus. The spores are consistently wider and usually longer than in *G. sideroides* and the gelatinous subhymenium is very distinct.

6. **Galerina farinosipes** sp. nov.

Pileus 1–2 cm latus, ovalis vel convexus, canescens, pallide fulvus; lamellae late adnatae, secedentes, confertae vel subdistantes; stipes 4–6 cm longus, 2–4 mm crassus, subtomentosus, deorsum fusco-brunneus; sporae 9–12 × 6–7 μ; pleurocystidia ventricose-subcapitata vel cylindrico-capitata, 44–56 × 9–12 μ; cheilocystidia 40–65(–75) × 8–10 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum: legit in terra prope Trinidad, Calif., Nov. 29, 1935, Smith n. *3649*.

Pileus 1–2 cm broad, oval to convex young, the margin straight, expanding to campanulate to broadly convex, surface at first conspicuously white-hoary from a dense coating of fine white fibrils, margin usually decorated with patches of the thin veil, glabrescent, surface moist, hygrophanous, "ochraceous tawny" and faintly striate to opaque on the margin, fading to near "warm buff"; flesh rather thick and firm, near ochraceous tawny and fading to buff, odor and taste not distinctive; lamellae moderately broad, adnate with a decurrent line, soon seceding, close to subdistant, pallid brown becoming ochraceous tawny, edges white floccose; stipe 4–6 cm long, 2–4 mm thick, equal, hollow, cartilaginous-fragile, usually somewhat twisted, densely pruinose-tomentose over all from a

dense grayish white tomentum, interior dark brown to bister in basal part, yellowish above.

Spores 9–12 × 6–7 μ, inequilateral in side view, ovate in face view, ochraceous tawny or paler in KOH, nearly smooth (as seen under oil minutely warty-rugulose), with a ragged line marking the suprahilar depression, apical pore if present very minute; basidia two-spored, 26–32 × 7–8 μ, hyaline; pleurocystidia scattered to abundant, 44–56 × 9–12 μ, ventricose-subcapitate to subcylindric-capitate or at least apices broadly rounded, hyaline, thin-walled; cheilocystidia abundant, 40–65(–75) × 8–10 μ, cylindric-subcapitate or the elongated part flexuous, hyaline and thin-walled; gill trama interwoven, yellowish in KOH; pileus trama homogeneous, yellowish in KOH, clamp connections present but rare, many cylindric-subcapitate pilocystidia arising from the cap surface, these cells 100–300 × 7–10 μ (these often appearing to form a nongelatinous pellicle).

Habit, habitat and distribution: Gregarious on soil, Trinidad, Calif., Nov. 29, 1935, coll. H. E. Parks (Smith-*3649*).

Observations: This species is grouped with *G. pruinatipes* because of the broadly rounded pleurocystidia and cheilocystidia and the presence of pilocystidia. Actually, however, the two species are quite different in appearance. *G. farinosipes* has a rather heavy sheath of white veil fibrils over the lower part of the stipe. The pilocystidia cause the white-hoary appearance of young caps. In age these elements are considerably elongated and matted down into a layer that can easily be mistaken for a pellicle if only cross sections of the gills and pileus are cut. The spores of *G. farinosipes* are scarcely roughened (as seen under oil) but the suprahilar depression is clearly bounded by a ragged line. The spores of *G. pruinatipes* are, relatively speaking, conspicuously roughened.

7. **Galerina ferruginea** sp. nov.

Pileus 8–12 mm latus, conicus demum late conicus vel campanulatus, interdum convexus, latericius; sapor farinaceus; lamellae latae, ventricosae, subdistantes, adnatae, secedentes; stipes 2–3.5 cm longus, 1–1.5 mm crassus, deorsum ferrugineus, sparsim fibrillosus; sporae 8–10(–11) × 4–5 μ; cheilocystidia 26–34(–46) × 6–10 μ, fusoidi-ventricosa et subcapitata; carbonicola. Specimen typicum in Herb. Mich. conservatum; legit prope Whitmore Lake, Mich., Sept. 25, 1933, Smith n. *33-1016*.

Pileus 8–12 broad at base, conic with a straight or connivent margin young, expanding to broadly conic, campanulate or convex

at maturity, glabrous, moist "*Hays russet*" to "*cinnamon rufous*" or "*ferruginous*," subhygrophanous and reddish tan faded, when moist only faintly translucent-striate; flesh thin, concolorous with surface, *odor and taste slightly farinaceous;* lamellae broad, becoming ventricose, subdistant, adnate, seceding, pale brown at first, ochraceous orange at maturity, edges even to irregular; stipe 2–3.5 cm long, 1–1.5 mm thick, equal or enlarged above, tubular, lower half concolorous with pileus, upper portion paler, glabrous except for scattered fibrils over lower portion representing the remains of the rudimentary veil.

Spores 8–10(–11) × 4–5 μ, inequilateral in side view, ovate in face view, tawny or darker in KOH, minutely tuberculate, exospore loosening somewhat over suprahilar area but that area not entirely smooth, apical pore minute; basidia four spored, hyaline in KOH, 23–26 × 6–7 μ; pleurocystidia none; cheilocystidia abundant, 26–34(–46) × 7–10 μ, fusoid-ventricose with wavy necks and obtuse to capitate apices, thin-walled, hyaline in KOH; gill trama subparallel to interwoven, dull yellow (near ocher yellow) in KOH from incrusting pigment, gradually fading out to pallid, pileus trama homogeneous, yellow in KOH but fading out to pallid as in gill trama, clamp connections present.

Habit, habitat and distribution: Gregarious on burned peat, Mud Lake Bog, Whitmore Lake, Mich., Sept. 25, 1933, Sm-*33–1016.*

Observations: The curious feature of this species is the manner in which particles of the outer layer of the spore wall adhere over the suprahilar depression. As a result, the boundary of the depression varies greatly in position from spore to spore, and the plage also varies in size and degree of irregularity. The fact that there is a tendency for the "plage" area to become smooth is here considered as a valid reason for referring the fungus to *Galerina.* The truly ferruginous red color and farinaceous taste as well as the habitat aid in distinguishing the species in the field.

8. **Galerina fibrillosa** sp. nov.

Pileus 10–12 mm latus, plano-umbonatus, siccus, fibrillosus, obscure vinaceo-brunneus; lamellae latæ, confertae, adnatae, obscure vinaceo-brunneae; stipes 1–2 cm longus, 1–1.5 mm crassus, peronato-fibrillosus fibrillae vinaceo-brunneae; sporae 10–14 × 5.5–6.8 μ; cheilocystidia 40–60 × 10–14 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Gobler's Knob, Mt. Rainier National Park, Wash., Oct. 12, 1952, Smith n. *40850.*

Pileus 10–12 mm broad, obtuse when young, expanding to plano-umbonate or umbo obsolete, surface dry and coarsely matted-fibrillose or in buttons hairy fibrillose, "vernona brown" to "snuff brown" (fibrils appearing coarse under a lens); flesh thin and soft, bister in KOH when fresh, no distinctive odor or taste; lamellae broad, close, ventricose in age, adnate, "Verona brown" young, and in age ± concolorous with the pileus, edges minutely fimbriate from cheilocystidia (use a lens); stipe 1–2 cm long, 1–1.5 mm at apex, slightly thicker downward, fragile, dull brownish beneath a hairy-fibrillose coating similar to that on pileus and covering the lower two thirds, apex pruinose in age and lower portion ± glabrescent.

Spores dull ferruginous in deposits, dull cinnamon in KOH, 10–14 × 5.5–6.8 μ, inequilateral in side view, in face view narrowly ovate, smooth except for a rough border around the supra-hilar depression; basidia four-spored; pleurocystidia none; cheilocystidia 40–60 × 10–14 μ fusoid-ventricose, often with wavy necks and obtuse to slightly enlarged apices; gill trama somewhat interwoven, dingy cinnamon in KOH; pileus trama with a cuticle of loosely arranged crooked hyphae heavily banded with dull cinnamon, incrusted pigment, tissue beneath this layer paler cinnamon and more compactly interwoven; clamp connections present.

Habit, habitat and distribution: Gregarious on wet earth, Gobler's Knob, Mt. Rainier National Park, Wash., Oct. 12, 1952, Sm-*10850*.

Observations: This species is most closely related to *G. insignis* but differs in its darker color, more coarsely matted-fibrillose pileus, the darker gills, and the more well developed dark brown fibrillose covering of the stipe. The spores and cheilocystidia are somewhat similar in both. The fruiting bodies are not at all "*Galerina*-like" in appearance, but the rough boundary of the suprahilar depression would seem to exclude *Phacomarasmius*, where they would otherwise go.

9. **Galerina fuscobrunnea** sp. nov.

Pileus 5–10 latus, conicus, fusco-brunneus; lamellae subdistantes, latae, adnatae; stipes 2–3 cm longus, 1 mm crassus, fusco-brunneus; sporae 7.5–9.5(–10.4) × 4.5–5.5 μ, anguste inaequilateralis; cheilocystidia 32–48 × 5–8 μ, anguste clavata, sparsim incrustata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram in paludibus, prope Waupantia Summit, Cascade Mts., Ore., 3500 ft. alt., Sept. 24, 1946, Smith n. *23701*.

Pileus 5–10 mm broad, conic with a straight margin, expanding to conic-campanulate, glabrous, moist, hygrophanous, disc near "mummy brown" at first, "buckthorn brown" and striate over marginal area when mature, fading to pale buff on disc first; flesh concolorous with surface, soft, odor and taste mild; lamellae broadly adnate, subdistant, broad, 1–2 tiers of lamellulae, pale yellowish brown (± honey color); stipe 2–3 cm long, 1 mm thick, tubular, equal, concolorous with cap or a shade paler and with a few inconspicuous fibrils indicating presence of a thin veil.

Spores 7.5–9.5(–10.4) × 4.5–5.5 μ, narrowly subelliptic to very slightly inequilateral in side view, *narrowly* ovate in face view, smooth to very minutely roughened (exospore not separating from endospore), tawny or darker in KOH, suprahilar plate smooth but very indistinct and faintly bounded as seen under oil, apical pore not visible; basidia four-spored, hyaline in KOH; pleurocystidia none; cheilocystidia abundant, 32–48 × 5–8 μ, narrowly clavate to subcylindric, often with flexuous walls, apices of some with slight incrustation, hyaline in KOH, thin-walled; gill trama interwoven, rusty brown in KOH; pileus trama homogeneous, rusty brown in KOH, clamp connections present.

Habit, habitat and distribution: Gregarious on humus under alder, swampy place below Waupanitia Summit, Cascade Mts., Ore., 3500 ft., Sept. 24, 1946, Sm-*23701*.

Observations: The narrow, practically smooth, spores are distinctive along with the cheilocystidia. The habitat in alpine swamps may also be significant along with the very dark brown color of young carpophores.

10. **Galerina humicola** sp. nov.

Pileus 10–15 mm latus, conico-campanulatus, demum plano-umbonatus, glabrescens, cinnamomeo-brunneus; lamellae confertae, adnatae, secedentes, latae; stipes 1.5–2 cm longus, c. 1 mm crassus, deorsum demum badio-umbrinus, sursum fibrilloso-annulatus; annulus albus; sporae 5.5–6.3 × 4–4.5 μ; cheilocystidia 32–48 × 5.8 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram prope Milford, Mich., June 5, 1951, Smith n. *15552*.

Pileus 10–15 mm broad, conic-campanulate, the margin only slightly incurved at first and soon straight, expanding to plane with a sharp conic umbo, surface moist, at first with scattered fibrils from the remains of the veil, soon glabrous, translucent-striate, "cinnamon brown" to "ochraceous tawny" at first, hygrophanous and "warm buff" (yellow) faded, fading on disc first;

flesh thin, concolorous with surface, very soft and brittle, odor and taste slightly pungent; lamellae close (15–18 reach the stipe), 2 tiers of lamellulae, adnate but seceding readily, broad (± 2 mm), dull ochraceous tawny over all, edges even; stipe 1.5–2 cm long, ± 1 mm thick, slightly enlarged at apex, concolorous with pileus at first but soon becoming dark sordid brown from the base upward, *with a superior whitish fibrillose zone from the remains of the veil*, zone evanescent, sparsely white-fibrillose toward the base.

Spores 5.5–6.3 × 4–4.5 μ, inequilateral in side view, in face view subelliptic to ovate, tawny to pale russet in KOH, exospore wrinkled to rugulose-tuberculate, suprahilar depression present and apparently nearly smooth, apical pore present but minute; basidia four-spored; 16–19 × 5–6.5 μ, hyaline in KOH; pleurocystidia none seen; cheilocystidia 32–48 × 5–8 μ, abundant, fusoid-ventricose to almost tibiform (narrowly ventricose with subcapitate apices), hyaline, thin-walled (a few with yellowish content as revived in KOH); gill trama interwoven to subparallel, dull yellow-brown as revived in KOH; pileus trama homogeneous, no pellicle, dull yellow brown in KOH from incrusting pigment; clamp connections present at base of basidium.

Habit, habitat and distribution: Subcespitose on black muck near a stump, Milford, Michigan, June 5, 1941, Sm-*15552*.

Observations: This species differs from *G. latifolia* in having an annular zone of veil fibrils, spores which lack blisters of the loosened outer layer, and in its habitat on muck instead of oak wood. I at first considered describing it as a variety of *G. triscopa*, thinking that in *G. triscopa*, *G. laticeps* and *G. humicola* we had a situation comparable to that of the varieties in *G. badipes*. However, *G. laticeps*, on the basis of what we know so far, is distinct on spore characters and hence does not fit into such a series. As for *G. humicola*, we have no intergrading series to connect it with *G. triscopa* in the characters mentioned, and in addition its spores appear to be shorter *and* broader than those of *G. triscopa*.

11. **Galerina insignis** sp. nov.

Pileus 8–25 mm latus, late conicus, siccus, fibrillosus, pallide argillaceus; lamellae latae, confertae, cinnamomeae; stipes 2–3.5 cm longus, 1–1.5 mm crassus, deorsum brunnescens, peronato-fibrillosus; fibrae argillaceae; sporae 12–15 × 5–6.3 μ; cheilocystidia 36–48 × 8–11 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41067*.

Pileus 8–25 mm broad, obtusely conic expanding to broadly conic or margin spreading and umbo obtuse, surface dry and fibrillose, near "cinnamon buff," in age paler and near pinkish buff; flesh moist, *exceedingly* fragile, odor slight, taste slightly of raw cucumber; lamellae broad, ascending adnate, close, pinkish buff young, "Sayal brown" (cinnamon) at maturity, edges fimbriate; stipe 2–3.5 cm long, 1–1.5 mm thick, equal, lower part copiously fibrillose with "cinnamon buff" fibrils, pallid and silky to pruinose above, ground color brownish, base darker in age on some specimens.

Spores 12–15 × 5–6.3 μ, narrowly oblong in face view, obscurely oblong-inequilateral in side view, dull cinnamon in KOH; basidia four-spored; pleurocystidia scattered to rare, 40–56 × 10–13 μ, fusoid-ventricose with obtuse to slightly enlarged apices, hyaline, thin-walled, smooth; cheilocystidia mostly ventricose-capitate, 36–48 × 8–11 μ, head often as broad as the ventricose part; gill trama interwoven, ochraceous brown from encrusted pigment (darker in KOH); pileus trama of two ± equal parts, the upper of loosely arranged, widely spaced, heavily encrusted (dark cinnamon) hyphae 5–10 μ in diam., lower portion compactly interwoven and more ochraceous; clamp connections present.

Habit, habitat and distribution: Gregarious on soil in an old pile of debris from an avalanche, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Sm-*41067*.

Observations: The spores and cystidia are those of a *Galerina*, but the duplex structure of the pileus is not typical of any genus known to me. The suprahilar depression is seen to be marked off faintly when viewed under a good oil immersion lens, but the outer spore layer is practically smooth also. In optical section the subapical thickening of the outer wall gives the impression of a pair of earmuffs. The thickening extends clear around the spore, as can be perceived by noting the slightly darker color-band in this region.

12. **Galerina lacustris** sp. nov.

Pileus 10–15 mm latus, campanulátus, glaber, subviscidus, nitens, fulvus; lamellae latae, confertae vel subdistantes, adnatae, pallide argillaceae; stipes 4–6 cm. longus, 2–3 mm crassus, mollis, flexuosus, sparsim fibrillosus; sporae 10–12.5 × 5–6.3 μ; cheilocystidia 38–60 × 8–11 μ, clavata vel subventricosa et subcapitata, sparsim incrustata. Specimen typicum in Herb. Univ. Mich. conservatum: legit in paludibus, Lake George, Mt. Rainier National Park, Wash., Oct. 12, 1952, Smith n. *40848*.

Pileus 10–15 mm broad, obtusely conic, expanding to broadly campanulate, at times umbo obsolete and cap convex, surface glabrous, *lubricous* to *viscid* to touch when wet, *shining*, striate to disc, moist, bright "ochraceous tawny," disc a little darker; flesh soft and fragile (but fleshy for the genus), concolorous with surface, odor and taste mild; lamellae *broad*, close to subdistant, broadly adnate and readily seceding, "cinnamon buff" at maturity (paler than cap and duller), paler when young, edges even; stipe 4–6 cm long, 2–3 mm at apex, equal, *very soft and fragile*, hollow, at first with a few faint appresed fibrils below, apex faintly pruinose, concolorous with margin of cap over all or in some apex a little paler but no true color change present, *often translucent* and *undulating*.

Spores 10–12.5 × 5–6.3 μ, elongate-inequilateral in side view, narrowly ovate in face view, smooth except for a ragged line around the depression, *ochraceous tawny* in KOH; basidia four-spored; pleurocystidia none seen; cheilocystidia abundant, 38–60 × 8–11 μ, mostly clavate with flexuous pedicels but some ± ventricose at base and some ± filamentose, apical enlargement often oval, often somewhat incrusted beneath the apical enlargement; gill trama somewhat interwoven, ochraceous in KOH; pileus trama with a thin poorly formed subgelatinous pellicle (in KOH), main tramal body ochraceous tawny and homogeneous (in KOH); clamp connections present.

Habit, habitat and distribution: Scattered among mosses and sedges at low water level of Lake George, Mt. Rainier National Park, Wash., Oct. 12, 1952, Sm-*40848*.

Observations: The incrustation over the upper part of the necks of the cheilocystidia is peculiar in this genus. The line marking the suprahilar depression of the spore is very faint but can be observed on most properly oriented spores.

13. **Galerina laticeps** sp. nov.

Pileus 6–12 mm latus, convexus, dilute cinnamomeus, amarus; lamellae confertae, 1.5–2 mm latae, crassiusculae; stipes 1.2–5 cm longus, 1.5–2 mm crassus, aequalis, cavus, dilute cinnamomeus; sporae 7–8 × 5–6 μ; cheilocystidia 30–46 × 7–9 μ. Specimen typicum in Herb. Univ. Mich. conservatum; legit in ligno Pseudotsugae, Rhododendron, Ore., Nov. 17, 1944, Smith n. *19865*.

Pileus 6–12 mm broad, convex to obtusely conic at first, broadly convex in age, margin apparently straight at first, surface glabrous, moist, hygrophanous, with a very faint fringe of fibrils along the

margin when young, evenly "Sayal brown" and only faintly striate in age before fading, fading to sordid cinnamon buff or paler; flesh thin, brittle, concolor, odor none, taste bitter; lamellae close to crowded, 1–2 tiers, depressed to rounded adnate, moderately broad, ventricose (1.5–2 mm), thickish, faces concolorous with cap, edges whitish; stipe 1–2.5 cm long, 1.5–2 mm thick, equal, hollow, concolorous with or slightly paler than the pileus, not darkening below, at first with a faint fringe of white fibrils from the remains of a thin veil, soon glabrescent, base not conspicuously mycelioid.

Spores 7–8 × 5–6 μ, ovate to almost angular-ovate in face view, in side view inequilateral, with a smooth suprahilar depression, endospore thick and tawny to near russet in KOH, outer layer loosely enveloping the endospore except for suprahilar depression and very wrinkled to rugulose as well as somewhat deciduous (if mount is crushed in KOH), apical pore indistinct under oil; basidia four-spored, hyaline in KOH; pleurocystidia lacking; cheilocystidia scattered, 30–46 × 7–9 μ, thin-walled, hyaline in KOH, slightly ventricose at base, with a narrow neck and an obtuse to subcapitate apex, or filamentous-subcapitate; gill trama homogeneous, rather dark ochraceous tawny from incrusting pigment; clamp connections present—but most appear to be false clamps with no fusion of the clamp with the main hypha; pilocystidia similar to cheilocystidia, scattered to rare.

Habit, habitat and distribution: Gregarious on Douglas Fir log, Rhododendron, Oregon, Nov. 17, 1944, Sm-*19865* and *20143*.

Observations: The obtuse to convex pileus and relatively pale cinnamon pileus distinguishes this species from *G. triscopa* in the field, and the broader spores (almost angular-ovate in face view), distinguish it in the herbarium. A somewhat similar fungus occurs on hardwood in which pleurocystidia are present and in which a veil is absent. However, in it the distribution of pleurocystidia was quite erratic and more collections are needed to clarify the situation.

14. **Galerina occidentalis** sp. nov.

Pileus 10–18 mm latus, convexus, fulvus, *amarus;* lamellae confertae, angustae, cinnamomeae; stipes 2–3 cm longus, 2.5 mm crassus, subaequalis, fulvus, deorsum demum badius, sursum subannulatus; sporae 9–12 × 5.5–6.3 μ; cheilocystidia 26–34 × 6–10 × ± 2 × 3–4 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; ad terram, prope

Carbon River Entrance, Mt. Rainier National Park, Wash., Oct. 2, 1952, Smith n. *40441*.

Pileus 10–18 mm broad, obtuse to convex, expanding to plane or with a slight umbo, surface glabrous, moist, hygrophanous, *dark rusty cinnamon* to near tawny moist, fading to tan, striate before fading, fading to dingy cinnamon; flesh thin, concolorous with surface; taste *disagreeable-rancid*; lamellae horizontal, close, adnate, narrow to moderately broad, cinnamon, edges even to slightly fimbriate; stipe 2–3 cm long, up to 2.5 mm thick at apex, equal or enlarged downward, concolorous with or slightly paler than pileus, darkening below in drying, annulate above with a zone of fibrils, apex pruinose, lower portion sheathed with a thin white fibrillose coating, annulus at times evanescent.

Spores (8.5–)9–12 × 5.5–6.3 μ, ovate in face view, slightly inequilateral in side view, smooth or very indistinctly roughened, the depression smooth but boundary faint, tawny in KOH; basidia 2-spored; pleurocystidia none; cheilocystidia 6–10 μ broad at base and 26–34 μ long, *neck ± 2 μ thick* and often ending in a small capitelum (3–4 μ) with an apical thickening of the wall; gill trama parallel, cells enlarged and dark cinnamon in KOH; pileus trama homogeneous and dark cinnamon beneath a paler nongelatinous pellicle of narrower (4–6 μ) hyphae; clamp connections present.

Habit, habitat and distribution: On humus, Carbon River Entrance, Mt. Rainier National Park, Oct. 2, 1952, Sm-*40441*-type. An earlier collection, Sm-*30035*, was made in the Park Aug. 9, 1948 on wood at Lower Tahoma Creek.

Observations: *G. occidentalis* is closely related to *G. tahquamenonensis* but can be distinguished in the field by its very disagreeable taste and close, narrow lamellae. The variation in the habitat from lignicolous to "terrestrial" in *G. occidentalis* is not likely to mean much because of the great amount of lignicolous material in the soil in this area. The dark color of the pileus and gill trama, as well as their structure, is typical of *Psilocybe* but the spores preclude assignment to that genus. The simple structure of the cuticle of the pileus prevents *G. occidentalis* from being placed in *Alnicola*—where the spore characters of color and markings would be aberrant. The cheilocystidia, however, are reminiscent of that genus. Under the best oil immersion lens available it was clear that the pattern of markings on the spores was typical of *Galerina*.

15. **Galerina odora** sp. nov.

Pileus 1–3 cm latus, late conicus, glaber, subfulvus, odoratus (subfarinaceus); lamellae latae, confertae, adnatae, pallide argillaceae; stipes 3–6 cm longus, 2–3.5 mm crassus, deorsum sparsim fibrilosus; sporae 10–14 × 6.5–8 μ; cheilocystidia 36–50 × 7–11 μ. Specimen typicum in Herb. Univ. Mich. conservatum; legit St. Andrews Creek, Mt. Rainier National Park, Wash., Sept. 24, 1952, Smith n. *40210*.

Pileus 1–3 cm broad, obtusely conic, expanding to broadly conic or somewhat umbonate, surface glabrous, moist and hygrophanous, faintly striate, dark ochraceous tawny moist, fading to cinnamon buff; flesh buff color, thick in the disc, thin over margin, odor resembling that of fresh green corn, taste mild; lamellae broad, close, adnate, cinnamon-buff becoming ochraceous tawny or darker, edges white fimbriate; stipe 3–6 cm long, 2–3.5 mm at apex, evenly enlarged downward, lower part coated with appressed pallid fibrils or these in zones or patches, apex pruinose, brownish downward but when dried evenly reddish brown overall.

Spores 10–14 × 6.5–8 μ, ovate in face view, in side view inequilateral, with blisters of the loosened exosporium especially around the smooth suprahilar depression; basidia four-spored, 24–30 × 9–10 μ, with globular content; pleurocystidia none; cheilocystidia abundant, 36–50 × 7–11 μ, narrowly ventricose with obtuse to slightly enlarged apices or in some apex tapered rather abruptly; gill trama regular, cells greatly enlarged, pale ochraceous in KOH; subhymenium appearing cellular in sections; pileus trama with a thin pellicle of narrow, ochraceous, non-gelatinous hyphae, tissue beneath of broad, interwoven ochraceous hyphae; clamp connections present.

Habit, habitat and distribution: Gregarious on a creek bank, St. Andrews Creek, Mt. Rainier National Park, Wash., Sept. 24, 1952, Sm-*40210*.

Observations: *G. odora* is distinct from *G. cortinarioides* by virtue of the odor of fresh green corn when the flesh is crushed and by the larger spores. Like *G. cortinarioides*, *G. odora* is almost certain to be confused in the field with the small slender species of *Cortinarius*.

16. **Galerina olympiana** sp. nov.

Pileus 5–10 mm latus, convexus, margine primo involuto, demum planus, glaber; lamellae confertae vel subdistantes, angustae, crassiusculae; stipes 1–2 cm longus, c. 1 mm crassus, flexuosus, deorsum badius et luteo-fibrillosus; sporae 8–10 × 4.5–5.5 μ (10–12 × 6–7 μ); basidia tetraspora vel bispora;

pleurocystidia 35–56(–60) × 12–15(–20)μ, apice late rotunda. Specimen typicum in Herb. Univ. Mich. conservatum: legit in ligno *Populi trichocarpi*, Hoh River, Olympic National Park, Wash., June 6, 1939, Smith n. *14116*.

Pileus 5–10 mm broad, convex with a slightly incurved margin, expanding to plane or nearly so, surface glabrous, hygrophanous, when fresh and moist "tawny" and opaque, becoming "ochraceous tawny" and conspicuously striate before fading to "warm buff" (pale yellow) or paler; flesh thick (for such a small fungus) and firm, concolorous with surface, odor and taste not distinctive; lamellae subdistant to close (13–15), narrow, horizontally adnate or at times slightly toothed, *rather thick*, concolorous with pileus and pruinose; stipe 1–2 cm long, about 1 mm thick, equal, crooked to undulating, with a narrow tubule, concolorous with pileus or darker reddish brown below, apex coarsely pruinose, lower portion with scattered patches of appressed *yellowish fibrils*.

Spores 8–10 × 4.5–5.5 μ (10–12 × 6–7 μ, 2-spored) ovate in face view, subinequilateral in side view, tawny as revived in KOH, pale yellow fresh, slightly rugulose-roughened, apical pore present but apex not truncate, suprahilar depression both slight and smooth; basidia 2- or 4-spored, 17–20 × 7–7.8 μ, clavate, slightly projecting; pleurocystidia abundant, 35–56(–60) × 12–15(–20)μ, thin-walled, hyaline or in some the content yellow when fresh, apices broadly rounded, lower part ventricose, pedicel slender and elongated; cheilocystidia occasionally with a globular hyaline inclusion, forming a sterile band on gill edge, more nearly cylindric than the pleurocystidia, apices broadly rounded, 38–56 × 9–15 μ; gill trama parallel, tawny in KOH from heavily incrusting pigment, clamp connections present; pileus trama tawny to russet in KOH, the incrusting pigment heavy, homogeneous, pellicle not differentiated, occasional pilocystidia similar to pleurocystidia present.

Habit, habitat and distribution: Gregarious on wood of *Populus trichocarpa*, Hoh River, Olympic National Park, Wash., June 6, 1939, Sm-*14116*.

Observations: Small metuloid cysts 6–8 μ broad and of variable length are also present in the hymenium. These do not appear to be typical gloeocystidia, but may be an additional distinctive feature of this very unusual little agaric. They are rare, and, since there is a strong tendency for both basidia and cystidia to have colored or granular-refractive content, these may simply be basidioles in a certain stage of development.

The distinguishing field characters are the small size, habitat on

cottonwood, thickish gills, and yellowish veil fibrils. Microscopically the pleurocystidia are most like those of *G. pruinatipes* but the markings on the spores are faint—as in *G. farinosipes*. In addition, there is more of a tendency for the cystidia to have a colored content than in either of the others. There are a large number of hyaline fusoid-ventricose caulocystidia (with obtuse apices) mixed with the capitate caulocystidia. *G. pruinatipes* var. *fulvipes* is a robust fungus. Both *G. pruinatipes* var. *pruinatipes* and var. *fulvipes* are specificaly distinct from *G. olympiana* by the markings on the spores.

17. **Galerina pallidispora** sp. nov.

Pileus 10–25 mm latus, campanulatus, "russet" demum fulvus; lamellae adnatae, confertae, latae; stipes 3–4 cm longus, 2–3 mm crassus, ± aequalis, "russet" vel fulvus, concolor, glaber, sursum pruinosus; sporae 9–13 × 5–6.5 μ, pallide ochraceae; cheilocystidia 24–32 × 6–9 μ, ventricosa et capitata. Specimen typicum in Herb. Mich. conservatum; legit in ligno Alni, Graves Creek, Olympic National Park, Wash., May 23, 1939, Smith n. *13694*.

Pileus 10–25 mm broad, obtusely conic expanding to campanulate, surface glabrous, moist, hygrophanous, "russet" at first but becoming "ochraceous tawny" before fading, "warm buff" faded and when dried pale buff; lamellae bluntly adnate, close, moderately broad, ochraceous tawny, edges even; stipe 3–4 cm long, 2–3 mm thick, equal or slightly enlarged downward, concolorous with cap and fading like it, not darkening in age or on drying, pruinose above, glabrous below except for mycelium around base.

Spores 9–13 × 5–6.5 μ, subinequilateral to nearly oblong in side view, narrowly ovate to suboblong in face view, *pale ochraceous* in KOH, surface minutely rugulose-warty and suprahilar plate faintly delimited, apical pore minute; basidia two-spored, 24–28 × 6–7.5 μ, hyaline; pleurocystidia none seen; cheilocystidia ventricose-capitate to ventricose with narrow necks, apices of some slightly incrusted or with mucilage cap, 24–32 × 6–9 μ, thin-walled, hyaline; gill trama parallel or nearly so, tawny to yellow in KOH; subhymenium not distinctive; pileus trama homogeneous, clamp connections present.

Habit, habitat and distribution: Scattered on alder logs, Graves Creek, Olympic National Park, Wash., May 23, 1939, Sm-*13694*.

Observations: The pale ochraceous spores, lack of pleurocystidia, small cheilocystidia (many with capitate apices), russet pileus and unicolorous stipe distinguish this species. The cheilocystidia and

aspect of the carpophore resemble those of *G. sideroides*, but the slightly roughened spores do not allow it to be placed in that group.

18. **Galerina pruinatipes** sp. nov.

Pileus 10–20 mm latus, obtusus vel convexus, demum planus, flulvo-cinnamomeus, striatus, fragilis, amarus; lamellae latae, confertae, adnatae, secedentes, acie albido-fimbriatae; stipes 1–3 cm longus, c. 1 mm crassus, fragilis, deorsum badius, sursum pruinosus; sporae 10–12.5 × 6.3–8 μ; pleurocystidia 40–55 × 10–18 μ, ventricosa, capitata vel subcapitata; pilocystidia cheilocystidia similia; cheilocystidia 36–44 × 9–13 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. of Mich. conservatum; legit in ligno coniferarum Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41029*.

Pileus 10–15(–20) mm broad, obtuse, expanding to plane, surface glabrous, moist, hygrophanous, rich tawny fading to pinkish buff (±), translucent striate moist; flesh very delicate and fragile, odor none, taste bitter; lamellae close, broad, bluntly adnate and readily seceding, pale ochraceous tawny young, between cinnamon buff and "cinnamon" mature, edges crenulate; stipe 1–3 cm long, ± 1 mm thick, equal except for slight bulb at base, fragile, *dark* reddish brown below, pallid and coarsely pruinose above, with *scattered pallid fibrils* downward, *base with a slight tawny pubescence*.

Spores 10–12.5 × 6.3–8 μ, inequilateral in side view, in face view ovate, warty-roughened and outer layer separable around the smooth depression, near russet in KOH; basidia two-spored; pleurocystidia scattered, 40–55 × 10–18 μ, ventricose with broadly rounded to capitate apices, hyaline, thin-walled; cheilocystidia 36–44 × 9–13 μ, mostly cylindric-capitate but some fusoid-ventricose elements present; gill trama of enlarged hyphal cells ± ochraceous in KOH, subregular; pileus trama homogeneous, elements mostly enlarged but some hyphae 5–10 μ in diam. present over surface, pigment incrusted; clamp connections present; pilocystidia similar to cheilocystidia or with somewhat colored walls; caulocystidia similar to cheilocystidia but in general more variable in shape, dull cinnamon to hyaline in KOH, many projecting filaments also present.

Habit, habitat and distribution: Scattered on rotten wet conifer logs in cold places, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41029*.

Observations: The broadly rounded to capitate pleurocystidia, cheilocystidia, pilocystidia and caulocystidia along with the dark-

colored, rough spores with their conspicuous suprahilar depression are distinctive. The stipe as revived in KOH becomes very dark. There is considerable variation in the cheilocystidia. Some are fusoid-ventricose with merely obtuse apices whereas the other extreme is almost cylindric capitate. All those observed were hyaline or at the most only faintly ochraceous at the very base. The outstanding field characters are the obtuse to plane pileus, coarsely pruinose stipe over upper half, pallid fibrils over lower half, the darkening of the stipe from the base up, the *tawny mycelium matted* around the base, and the bitter taste.

GALERINA PRUINATIPES var. **fulvipes** var. nov.

Pileus 1.5–3 cm latus, obtuse conicus demum subcampanulatus vel planoumbonatus; stipes 5–6 cm longus, c. 2 mm crassus, subaequalis, deorsum fulvo-fibrillosus; sporae 10–12.5 × 5.5–7 μ; pleurocystidia 38–52 × 12–18 μ, ventricosa et capitata; cheilocystidia 40–65 × 9–15 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit Jackson Guard Station, Hoh River, Olympic National Park, Wash., May 13, 1939. Smith n. *13398*.

Pileus 1.5–3 cm broad, obtusely conic becoming broadly conic or nearly plane with an obtuse umbo, glabrous, "tawny" when moist, translucent striate, hygrophanous and fading to "ochraceous buff"; flesh concolorous with surface, thin but not fragile, odor not distinctive, taste *bitter*; lamellae close (± 20 reach the stipe) narrow to moderately broad (± 3 mm), squarely adnate but soon seceding, nearly equal but broadest near stipe, dark ochraceous tawny, pruinose under a lens, edges fimbriate; stipe 5–6 cm long, ± 2 mm thick, slightly enlarged at base, dark reddish brown over all (all mature), *surface covered to near apex with dingy tawny appressed fibrils*, apex pruinose.

Spores 10–12.5 × 5.5–7 μ, slightly inequilateral in side view, in face view ovate, dark tawny in KOH, warty in KOH but suprahilar depression smooth, apical pore present but minute; basidia two-spored, 26–29 × 7–8.5 μ, hyaline in KOH; pleurocystidia present, 38–52 × 12–18 μ, ventricose with broadly rounded apices or apices capitate and as broad as the ventricose part, thin-walled and hyaline in KOH; cheilocystidia abundant, 40–65 × 9–15 μ, ventricose to cylindric below a capitate apex, broadest at apex, hyaline as revived in KOH or with a dull ochraceous to brown content; gill trama interwoven, pale ochraceous tawny in KOH from incrusting pigment; pileus trama darker than gill trama, homogeneous, clamp connections present; pilocystidia scattered,

similar to cheilocystidia or with slightly thickened ochraceous walls at least near base; caulocystidia in fascicles, ± like cheilocystidia but often more elongated, walls of some colored near base.

Habit, habitat and distribution: On conifer logs, Hoh River above Jackson Guard Station, Olympic National Park, Wash., May 13, 1939, Sm-*13398*.

Observations: This is a robust variety with tawny fibrils over lower half of stipe and with a tendency for many of the cheilocystidia to be colored. It may be a distinct species by virtue of the colored veil but I feel that more collections are needed to establish this.

19. **Galerina rudericola** sp. nov.

Pileus 10–25 mm latus, obtusus, margine primo involuto, demum plano-umbonatus, glaber; lamellae subdistantes vel distantes, subdecurrentes, angustae; stipes 3–5 cm longus, 1.5–2 mm crassus, aequalis, sursum fibrilloso-annulatus, deorsum demum fusco-brunneus; sporae 7–9 × 4–5 μ; pleurocystidia 40–50(–60) × 10–15(–18) μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41070*.

Pileus 10–25 mm broad, obtuse with an incurved margin young, expanding to plane with a conic umbo or the margin remaining decurved, surface glabrous, moist, "buckthorn brown" fading to near "pinkish buff" or pale pinkish buff, faintly striate moist; odor and taste not recorded; lamellae subdistant to distant, arcuate to short-decurrent, narrow, dingy ochraceous tawny, edges white-fimbriate; stipe 3–5 cm long, 1.5–2 mm thick, equal, fragile, with a submembranous to fibrillose superior annulus, punctate-fibrillose and buff colored above the ring, appressed fibrillose and dull honey color below, darkening from the base up in age.

Spores 7–9 × 4–5 μ, in side view obscurely inequilateral, in face view elliptic to narrowly ovate, minutely roughened and with a ragged line around the smooth depression but *no* blisters, color dark ochraceous tawny in KOH; basidia four-spored; pleurocystidia scattered, 40–50(–60) × 10–15(–18) μ, fusoid-ventricose, hyaline or with yellowish walls (in KOH), with obtuse apices but occasionally apex with a nipple-like protrusion either apical or to one side, or apex subcapitate; cheilocystidia similar to pleurocystidia or necks more flexuous and elongated; gill trama subparallel, subhymenium not distinctive; pileus trama homogeneous (no pellicle seen); clamp connections present.

Habit, habitat and distribution: On lignicolous debris at base of an old avalanche now well grown over the herbaceous plants and shrubs, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Sm-*41070*.

Observations: The fruiting bodies look like those of a very slender form of *G. marginata* but the fibrillose annulus and minutely asperulate spores with their smooth plage readily distinguish it. The colors of the pileus are dingy ocher brown, quite different from the typical ochraceous tawny to tawny of the *G. hypnorum* series. It differs sharply from *G. subochracea* in lacking a gelatinous pellicle or even a lubricous feel, as well as in the darkening stipe.

20. **Galerina rugisperma** sp. nov.

Pileus 8–15 mm latus, subplanus, pallide fulvus; lamellae latae, anguste adnatae; stipes 2–4 cm longus, c. 1 mm crassus, aequalis, deorsum badius; sporae 8–10 × 5–6 μ; cheilocystidia 38–60 × 8–13 μ, anguste ventricosa et capitata vel subcylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Longmire, Wash., Sept. 22, 1952, Smith n. *40146*.

Pileus 8–15 mm broad, obtuse, expanding to plane or nearly so, surface glabrous, moist, hygrophanous, striate, ochraceous tawny fading to pinkish buff but drying darker; lamellae broad, horizontal, narrowly adnate, edges slightly fimbriate, faces ochraceous tawny at maturity; stipe 2–4 cm long, ± 1 mm thick, equal, pale honey color above, becoming reddish brown from base up in age, veil thin and leaving scattered appressed patches of fibrils over lower portion.

Spores 8–10 × 5–6 μ, inequilateral in side view, in face view ovate, nearly russet in KOH, with a wrinkled outer layer variously loosened over the surface; basidia four-spored; pleurocystidia none; cheilocystidia abundant, 38–60 × 8–13 μ, ventricose to narrowly ventricose and with a capitellum 4–7 μ broad, or subcylindric-capitate, hyaline but as revived in KOH with a coloidal content; gill trama somewhat interwoven, ochraceous in KOH; pileus trama homogeneous, ochraceous in KOH; clamp connections present.

Habit, habitat and distribution: Gregarious on wet earth along edge of swamp, Longmire, Wash., Sept. 22, 1952, Sm-*40146*.

Observations: The large, capitate cheilocystidia, very loose episporium of the spore, darkening stipe, obtuse pileus and thin

veil are characteristic. In spore characters it is distinct from the *G. hyponorum* complex.

21. **Galerina subdecurrens** sp. nov.

Pileus 5–15 mm latus, late convexus, argillaceus, glaber; lamellae distantes vel subdistantes, subdecurrentes, latae; stipes 10–25 mm longus, 1 mm crassus, deorsum brunnescens et cinereo-fibrillosus; sporae 9–12.5 × 4–4.8 μ, leves; cheilocystidia 20–30 × 6–9 μ anguste ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Lower Tahoma Creek, Mt. Rainier National Park, Wash., Oct. 20, 1952, Smith n. *41225*.

Pileus 5–15 mm broad, obtuse to convex with young, expanding to broadly convex, surface glabrous, moist, hygrophanous, dingy ochraceous tawny to clay color moist, dingy pinkish buff faded, striate when moist, flesh thin and fragile, odor and taste not recorded; lamellae broad, subdistant to distant, broadly adnate to subdecurrent (approaching the condition found in *Xeromphalina picta*), seceding at times, edges even, dingy buff at first, ± ochraceous tawny when mature; stipe short, 10–25 × ± 1 mm, equal, pale honey color above, dark reddish brown below and lower half with pallid to grayish appressed fibrils (veil remnants?).

Spores (9–)10–12.5(–13) × 4–4.8 μ, narrowly oblong, some in face view narrowly ovate, *smooth*, pale cinnamon in KOH, and becoming pale ochraceous on standing, apical pore present but apex not truncate under ordinary magnifications, obscurely so under oil immersion; basidia four-spored, hyaline in KOH; pleurocystidia none; cheilocystidia fusoid ventricose, *small*, 20–30 × 6–9 μ, narrowly ventricose and apices obtuse; gill trama hyaline in KOH, somewhat interwoven; pileus trama homogeneous in structure but hyphae of upper half with ochraceous incrusting pigment; clamp connections present.

Habit, habitat and distribution: Cespitose-gregarious under alder on soil at edge of road, Lower Tahoma Creek (old camp ground), Mt. Rainier National Park, Oct. 20, 1952, Sm-*41225*.

Observations: The long, narrow, smooth, pale cinnamon to ochraceous spores, thin veil, and darkening stipe are distinctive. When viewed under oil a very faint ragged line can be made out delimiting the suprahilar area, but it cannot be demonstrated on all spores. I have demonstrated it under a 90 ×, 1.30 NA oil immersion objective. The species is thus a typical *Galerina* as interpreted here even though the apical pore is broader than usual

and reminds one of that found in *G. stagnina*. This type of apical pore occurring in a species in which the "plage" is still faintly delimited indicates the relationship of the *G. stagnina* stirps to the typical species of *Galerina*.

22. **Galerina subfiliformis** sp. nov.

Pileus 5–8 mm latus, 6–8 mm altus, acute conicus, dilute argillaceus, glaber; lamellae subdistantes, adnatae, latae; stipes 1–3 cm longus, subfiliformis, dilute ochraceus, sparsim fibrillosus; sporae 10–14 × 6–7 μ, *leves*; cheilocystidia 28–37 × 6–9 μ, fusoidi-ventricosa. Specimen typicum in ligni Tsagae, St. Andrew's Creek, Mt. Rainier National Park, Wash., Oct. 5, 1952, Smith n. *40607*.

Pileus 5–8 mm broad and 6–8 mm high, sharply conic, remaining unexpanded, surface glabrous, moist, hygrophanous, striate when moist, dingy yellowish to near cinnamon-buff moist, pale pinkish buff to whitish faded; odor and taste not recorded; lamellae broad, ascending adnate, subdistant to distant, pale ochraceous tawny in age; stipe 1–3 cm long, almost filiform (less than 1 mm thick) equal, fragile, very pale honey color over all, apex pruinose, base with pallid fibrils from the thin veil.

Spores 10–14 × 6–7 μ, pale ochraceous tawny in KOH, smooth, ovate in face view, obscurely inequilateral in side view; basidia four-spored; pleurocystidia none; cheilocystidia 28–37 × 6–9 μ, fusoid-ventricose with narrow necks and unenlarged apices; gill trama somewhat interwoven, hyaline in KOH; pileus trama homogeneous, hyaline to yellowish in KOH; clamp connections present.

Habit, habitat and distribution: Scattered on mossy conifer logs, St. Andrew's Creek, Mt. Rainier National Park, Wash., Oct. 5, 1952, Sm-*40607*.

Observations: The large, pale ochraceous tawny, smooth spores, pale pileus and stipe, and thin veil are distinctive along with the small conic cap. It fruits on wet logs in cold localities. It has the aspect of *G. hypnorum* but the smooth spores distinguish it.

23. **Galerina subochracea** sp. nov.

Pileus 6–15 mm latus, obtuse conicus demum late convexus, glaber, *subviscidus*, pallide fulvus; stipes 3–5 cm longus, 1–2 mm crassus, fragilis, luteus, fibrilloso-annulatus; sporae 8–9.5 × 4.5–5.2 μ; pleurocystidia 45–60 × 10–18 μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. of Mich. conservatum; legit prope Salmon River, Mt. Hood, Ore., Oct. 10, 1946, Smith n. *24347*.

Pileus 6–15 mm broad, obtusely conic to convex, expanding to plane, the margin connivent at first, surface lubricous to *viscid to the touch*, glabrous or with veil remnants scattered along the margin at first, hygrophanous, "ochraceous tawny" moist, fading to orange buff or paler; flesh thin and fragile, odor and taste not recorded; lamellae moderately broad, broadly adnate, close to subdistant, pale ochraceous tawny with white fimbriate edges; stipe 3–5 cm long, 1–2 mm thick, equal or nearly so, tubular, fragile, yellow over all at first and with a *distinct medium fibrillose annulus*, with scattered fibrils or patches below, somewhat darkening from base upward in age, but tending to fade again in drying.

Spores 8–9.5 × 4.5–5.2 μ, tawny ochraceous in KOH, inequilateral in side view, ovate in face view, exospore very minutely roughened, smooth suprahilar depression evident, pore apical and distinct; basidia 23–26 × 7–8 μ, four-spored, sterigmata very indistinct; *pleurocystidia abundant*, 45–66 × 10–18 μ, fusoid-ventricose with obtuse to subacute apices; cheilocystidia abundant, similar to pleurocystidia or slightly more variable in shape; gill trama parallel to somewhat interwoven, pale yellow to hyaline in KOH; pileus trama with a thin surface layer of hyphae 5–7 μ diam. with incrusted walls, these hyphae quite contorted and somewhat gelatinous, remainder of flesh homogeneous, yellow in KOH near pellicle, nearly hyaline to [illegible] hymenium; clamp connections present; no pilocystidia seen.

Habit, habitat and distribution: Scattered on debris at edge of beaver pond, East Fork of Salmon River, Mt. Hood, Oregon, October 10, 1946, coll. Wm. B. Gruber (Sm-*24347*). An additional collection referred here is Sm-*19904*, from debris near Rhododendron, Oregon, Oct. 18, 1944.

Observations: The diagnostic characters are the viscid cap, fibrillose annulus, small nearly smooth spores with a smooth suprahilar depression and conspicuous pleurocystidia. The convex to plane pileus is an additional field character. In the dried state the fruiting bodies are concolorous throughout. The darkening of the stipe, as noted on the fresh material, fades out in drying. Although related to *G. marginata* in spore and cystidial characters, one would be more inclined to class *G. subochracea* as a *Cortinarius* in the field. The smooth suprahilar depression of the spore, of course, makes a reference to *Cortinarius* untenable. Actually there are a number of characters which separate it from *G. marginata* and forms. The annulus is a fibrillose zone, the

pleurocystidia are shorter and more ventricose, the spores and tramal tissues paler in KOH, and the darkening of the stipe is not so pronounced and is not retained in drying. In one collection (Sm-*19904*) the pileus was merely lubricous, but in view of the situation in *G. unicolor*, as regards the pellicle, no emphasis is given the difference in viscidity in the two collections described as *G. subochracea*. The veil in collection *19904* dried yellowish, but notes on the color of the veil when fresh were not taken for either collection.

24. **Galerina tahquamenonensis** sp. nov.

Pileus 8–15 mm latus, campanulatus, subbadius, margine appendiculatus; lamellae subdistantes, latae, adnatae; stipes 1–2 cm longus, 1–1.5 mm crassus, subaequalis, deorsum subbadius, peronato-fibrillosus, sursum fibrilloso-annulatus; sporae 7–9(–10–12) × 5–6.5(–7) μ (basidia 1-, 2-, 3-, 4-spora); cheilocystidia 24–36 × 9–11 μ, ventricose et acuta vel subcapitata. Specimen typicum in Herb. Mich. conservatum; legit in ligno *Fagi*, Tahquamenon Falls State Park, Mich., July 2, 1952, Smith n. *39045*.

Pileus 8–12(–15) mm broad, obtusely conic with a ± incurved margin, expanding to campanulate or remaining convex, glabrous except for margin which is at first appendiculate with patches of the thin veil (± dentate-appendiculate), veil remnants soon evanescent, color when young and moist "Mars brown," soon "cinnamon brown" but the margin becoming dingy ochraceous tawny and striate, hygrophanous, fading to dingy cinnamon buff or paler; flesh concolorous with pileus, soft, taste and odor not distinctive; lamellae broadly adnate, broad, nearly subdistant at first, truly subdistant at maturity, ochraceous tawny (paler than pileus), edges even; stipe short, ± 1–2 cm long, ± 1–1.5 mm thick, equal or enlarged below, concolorous with pileus below, apex soon fading out to almost concolorous with the gills but in age again often darker, *peronate-fibrillose* up to the superior *thin often fragmentary annulus*, pruinose above, in age glabrescent.

Spores 7–9(–10–12) × 5–6.5(–7) μ, ovoid to subelliptic, *roughened at least around the suprahilar plate*, ochraceous tawny when revived in KOH; basidia 1–2–3–4-spored; *pleurocystidia* none seen; cheilocystidia 24–36 × 9–11 μ, ventricose with a narrow neck and sometimes with a small capitellum, wall of capitellum thickened in some and highly refractive; gill trama parallel, the cells short-ellipsoid, tawny to rusty brown in KOH, subhymenium not distinctive; pileus trama dark rusty brown in KOH and homogeneous, pigment incrusted on walls of hyphae; clamp connections present.

Habit, habitat and distribution: Gregarious on rotten hardwood, Tahquamenon Falls State Park, Michigan, July 2, 1952, Sm-*39045*. Sm-*37025*, July 23, 1951 and Sm-*38355*, Aug. 28, 1951 were also from the Park and on the same substratum.

Observations: No pilocystidia were observed. The stature of this species is that of *G. triscopoda* but the well-developed veil readily distinguishes it. The roughness of the spores is best seen under oil immersion. The smooth suprahilar depression is indistinctly bounded in most spores but can be seen in enough to verify its presence. The species appears to be most closely related to *G. camerina* sensu Kuhner (l.c., p. 213) in the characters of spores and cheilocystidia, but differs in having a peronate veil and usually in being annulate, and in growing on hardwood logs.

25. **Galerina umbrinipes** sp. nov.

Pileus 6–10 mm latus, conicus, demum campanulatus, glaber, pallide fulvus, striatus; lamellae adnatae, subdistantes, angustae demum latae; stipes 3–4 cm longus, 1–1.5 mm crassus, aequalis, *umbrinus;* sporae 10–12.5(–13) × 5.5–7 μ; pleurocystidia diversa: 1) 23–30 × 5–6 μ, fusoidi-ventricosa, 2) 46–80 × 9–14 μ, ventricosa et subcapitata. Specimen typicum in Herb. Mich conservatum; legit prope Still Creek, Mt. Hood National Forest, Oregon, Oct. 3, 1946, Smith n. *24051*.

Pileus 6–10 mm broad, obtusely conic, remaining conic or becoming campanulate, glabrous, moist, hygrophanous, "ochraceous tawny" moist, pale buff faded, when moist translucent-striate to the waters disc, somewhat sulcate faded; flesh very thin and fragile, no appreciable odor; lamellae ascending-adnate, narrow to moderately broad, 1 tier of lamellulae, subdistant, ochraceous tawny; stipe 3–4 cm long, 1–1.5 mm thick, equal or base slightly enlarged, *pale umber over all when young*, in age fading out to more or less concolorous with pileus in upper part, umber cast remaining at base, surface finely pubescent over all from projecting caulocystidia.

Spores 10–12.5(–13) × 5.5–7 μ, tawny to pale tawny in KOH, inequilateral in side view, ovate in face view, outer wall wrinkled, suprahilar area slightly roughened and not noticeably depressed, apical pore very minute, not readily visible except under oil; basidia four-spored, 23–26 × 6–7 μ, hyaline in KOH, not projecting when sporulating; pleurocystidia of two types, the first rare (possibly immature stages of second type) 23–30 × 5–6 μ, fusoid and imbedded in hymenium, hyaline and homogeneous in KOH, the second abundant, greatly elongated 46–80 × 9–14 μ, ventri-

cose at base, apex subcapitate to subacute, hyaline, thin-walled in KOH or walls slightly thickened; cheilocystidia similar to pleurocystidia, very abundant; gill trama subparallel, the cells short and broad, with ochraceous encrusting pigment on the walls as revived in KOH, yellow in water mounts of fresh material; pileus trama homogeneous, pellicle undifferentiated, cells more compactly interwoven near surface than they are deeper down, the walls yellow in KOH from encrusting pigment; caulocystidia fusoid ventricose, hyaline, thin-walled in KOH, (40–)50–100 × 12–18 μ, ventricose at base, tapered to a subcapitate to subacute apex.

Habit, habitat and distribution: Gregarious on moss under conifers, Still Creek Forest Camp, 3800 ft. elev., Mt. Hood National Forest, Washington, Oct. 3, 1946, Sm-*24051*.

Observations: The umber stipe is an unusual character in this genus, particularly on young specimens. It is likely that very small buttons have a similar cast to the pileus. The young stipe may appear almost fibrillose at times but an examination under the microscope shows the fibrils to be elongated caulocystidia. The spores are essentially as in *G. rubiginosa*, but the color of the stipe when young and the presence of small pleurocystidia along with the large ones are a pair of characters which prevent *G. umbrinipes* from being attached to *G. rubiginosa* as a taxon below the rank of species. The small pleurocystidia are indeed curious; when forked they resemble 2-spored basidia with thick, obtuse sterigmata.

26. **Galerina vaccinii** sp. nov.

Pileus 6–12(–15) mm latus, campanulatus, glaber, udus et striatus, sicco fibrillosus, subfulvus; lamellae confertae demum distantes, late adnatae, pallide argillaceae; stipes 10–20 mm longus, c. 1 mm crassus, aequalis, fragilis, deorsum brunnescens, sparsim fibrillosus; sporae 10–12.5 × 6–7 μ; cheilocystidia 38–50 × 9–12 μ, fusoidi-ventricosa vel subcylindrica. Specimen typicum in Herb. Univ. Mich. conservatum; legit sub *Vaccinio delicioso* prope Snow Lake, Mt. Rainier National Park, Wash., Oct. 11, 1952, Helen V. Smith (A. H. Smith n. *40798*).

Pileus 6–12(–15) mm broad, obtuse when young, becoming obtusely campanulate to plano-umbonate or umbo obsolete, dull tawny to dark ochraceous tawny and widely striate moist, near cinnamon buff faded, appearing glabrous when moist but when faded appearing fibrillose under a lens; flesh thin and very fragile, odor and taste not distinctive; lamellae close or finally distant,

broadly adnate or with a decurrent tooth, broad, *cinnamon-buff* when mature, paler when young, edges faintly fimbriate; stipe 10–20 mm long, ± 1 mm thick, equal, fragile, concolor with pileus but base darkening in age, thinly fibrillose over lower part from the pallid remnants of a veil, glabrescent.

Spores 10–12.5 × 6–7 μ elongate ovate in face view, obscurely elongate-inequilateral in side view, *smooth* but with a faint ragged line marking the boundary of the suprahilar depression, ochraceous tawny or slightly darker in KOH, in water mounts of fresh material pale ochraceous; basidia 4-spored; pleurocystidia none, a few ocher-brown basidioles present in hymenium; cheilocystidia 38–50 × 9–12 μ, fusoid-ventricose to subcylindric with obtuse apices, abundant; gill trama somewhat interwoven, ochraceous in KOH, the hyphal cells enlarged; pileus trama homogeneous but cells near surface enlarged giving almost the appearance of sphaerocysts, pilocystidia present as hyphal end-cells, trama rusty in KOH from heavy pigment incrustations; clamp connections present.

Habit, habitat and distribution: Cespitose-gregarious on duff under *Vaccinium deliciosum*, Snow Lake, Mt. Rainier National Park, Wash., Oct. 11, 1952, coll. Helen V. Smith (AHS-*40798*).

Observations: The spores are more like those of *Galerina* than *Cortinarius*, and since cheilocystidia are typical of the former, the collection is placed there. The dried specimens, however, remind one of the latter genus.

27. **Galerina venenata** sp. nov.

Pileus 1–3.5 latus, late convexus demum planus vel depressus, glaber, udus, cinnamomeus, hygrophanus, deinde pallidus; sapor farinaceus, demum amarus; lamellae latae, subdistantes, late adnatae, fulvae; stipes 3–4 cm longus, 3–5 mm crassus, deorsum sub-clavatus; anulatus; sporae 8–11 × 6–6.5 μ; pleurocystidia 45–70 × 9–15 μ; cheilocystidia pleurocystidiis similia. Specimen typimum in Herb. Univ. Mich. conservatum; lectum Portland, Ore., Jan., 1953.

Pileus 1–3.5 cm broad, broadly convex young, expanding to plane or with a slightly uplifted, arched margin and depressed disc, surface glabrous and moist, near cinnamon brown, hygrophanous, fading to a very dingy yellowish white to pinkish buff (often browner because of deposited spores), margin crenate to lacerated; flesh moderately thick, odor farinaceous, taste farinaceous but slowly becoming bitter and disagreeable, leaving an extended dryness in the throat; lamellae *broad*, subdistant, *broadly* adnate, golden tawny becoming dull cinnamon, edges even; stipe 3–4 cm

long, 3–5 mm at apex, enlarged somewhat toward the base, brownish, with a very thin, apical annulus appressed against the stipe in about half the specimens, glabrous except for the white-mycelioid base.

Spores 8–11 × 6–6.5 μ, subinequilateral in side view, ovate in face view, russet in KOH, with a conspicuously rugulose-warty outer wall tending to loosen to form blisters near hilar end, suprahilar depression smooth; basidia four-spored, content oily-globular, 20–25 × 7–8 μ; pleurocystidia present, 45–70 × 9–15 μ, hyaline in KOH, smooth, fusoid-ventricose with acute to subacute apices; cheilocystidia similar to pleurocystidia, no clavate to mucronate cells seen; gill trama somewhat interwoven, ochraceous in KOH, hyphal cells up to 30 μ broad, hymenopodium not distinctive; pileus trama homogeneous, the hyphae at the surface 6–12(–15) μ in diam. and interwoven, tramal body ochraceous in KOH; clamp connections present.

Habit, habitat and distribution: Gregarious on a lawn, Portland, Oregon, Jan. 24–27, 1953. Coll. by members of the Oregon Mycological Society.

Observations: This fungus was positively identified by a man and his wife as the fungus they ate a short time before being stricken with a severe case of fungous poisoning. The clinical history of this case is being published by Dr. Grossman of Portland, Ore.

There has been discussion of the edible and poisonous properties of the *Galerina marginata* complex in the literature (Harding, doctoral thesis, 1952). "*Pholiota autumnalis*" was blamed for a case in Minnesota, and Harding showed clearly that in this case a number of species of fungi (at least 2) had served to confuse the issue. As a result of his study he was inclined to blame a species of *Gymnopilus* as the poison species and cited work he had done on *Galerina marginata* which supported the conclusions of European authors that it is an edible fungus. He regarded *G. autumnalis* as a synonym of *G. marginata*.

The discovery of this poisonous fungus in Portland, Ore. reopens the whole matter for additional consideration. First, the possibility still exists that *Galerina autumnalis*, as recognized by us, may be a poisonous species. We do not dispute the claim that *G. marginata* is edible. That seems to be well established. How-

ever, from the data at hand we are forced to the conclusion that a second poisonous species in the group does exist, namely *G. venenata*. Since the latter has a very different cap cuticle from *G. autumnalis* we cannot consider it as a variety or form of Peck's fungus. In addition there is the difference in the shape of the apex of the cystidia and in the habitat. The very thin veil of *G. venenata* leaves only a most inconspicuous zone, and finally, the dried fruiting bodies do not compare at all favorably.

Because of the situation in regard to edibility in this group, we believe it is desirable to search for all characters which might be of use taxonomically in the hope of eventually being able to distinguish the poisonous from the edible members on correlated characters even though these may seem trivial in themselves. For this reason Smith and Singer have re-examined all the species placed in synonymy with *G. marginata* by Harding, and are recognizing them if there seems to be the slightest justification for doing so. We have examined the collection of the *Galerina* sent to Kauffman by Dr. Whetstone, and, though it is poorly dried, remains of a gelatinous pellicle were seen. Hence we classify this collection in *G. autumnalis*.

28. **Galerina vialis** sp. nov.

Pileus 1–4 cm latus, cinnamomeo-brunneus, glaber, hygrophanus, valde striatus; lamellae subdecurrentes, confertae vel subdistantes, subfulvae; stipes 3–5 cm longus, 2–4 mm crassus, deorsum valde brunnescens et peronato-fibrillosus, sursum annulatus; sporae 8.5–11 ×5–6.5 μ; pleurocystidia in KOH subochracea. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad viam prope Carbon River, Mt. Rainier National Park, Wash., Oct. 8, 1952, A. H. Smith n. *40709*.

Pileus 1–4 cm broad, convex when young, becoming plane or the disc shallowly depressed in age and margin arched, surface moist and hygrophanous, color evenly "cinnamon-brown" moist, gradually dingy ochraceous tawny before fading, fading on disc first to ochraceous buff but finally about pale pinkish buff translucent-striate nearly to disc when moist, glabrous but in age when faded ± squamulose from breaking of cuticle; flesh thin, soft, fragile, odor and taste not distinctive; lamellae broadly adnate to slightly decurrent, seceding, close to subdistant, broad, dull ochraceous tawny, edges minutely fimbriate; stipe 3–5 cm long, 2–4 mm at apex, narrowly clavate to nearly equal, concolorous with young gills above but base soon bister and discoloration progressing up-

ward, annulus superior, gray-fibrillose below annulus or fibrils breaking into squamules, coarsely pruinose-fibrillose above.

Spores 8.5–11 × 5–6.5 μ, in face view ovate and many with an almost snout-like apex, inequilateral in side view, rugulose-roughened and with "blisters" around the smooth suprahilar depression, russet in KOH; basidia 26–30 × 7.5–8 μ; pleurocystidia scattered 48–60 × 12–18 μ, fusoid-ventricose with subacute apices and the walls of the neck flexuous, thin-walled or walls slightly thickened, *dingy yellowish* to nearly hyaline in KOH, smooth; cheilocystidia abundant and fully as large or larger than the pleurocystidia, ventricose portion pale dingy yellow in KOH; gill trama subparallel, the cells ± ellipsoid, nearly hyaline in water when fresh, ochraceous to cinnamon-buff as revived in KOH; pileus trama homogeneous, the cuticular layer of nongelatinous hyphae, when revived in KOH the surface layer yellowish, a cinnamon colored layer beneath this and the remainder nearly hyaline; clamp connections present.

Habit, habitat and distribution: Gregarious-subcespitose along a road on soil, Carbon River, Mt. Rainier National Park, elev. 3300 ft., Oct. 8–9, 1952, Sm-*40709*, *40779*.

Observations: This species is in the *G. marginata* complex but is readily distinct when fresh by the dark brown pileus, more fragile consistency, and more conspicuous striae. Under the microscope the pale smoky-yellow cystidia are distinctive of revived material. Their walls are slightly thickened in the midportion and the yellow pigment is in the wall. As compared with *G. marginata* the fibrillose sheath over the lower portion of the stipe is much looser in organization, thicker, and in age leaves the lower part of the stipe with squamules or zones before becoming glabrous. Not much emphasis should be placed on the habitat being terrestrial because it is entirely possible that considerable wood has been used as a fill in making the road.

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MYXOMYCETES OF LOUISIANA

BERNARD LOWY

(WITH 10 FIGURES)

The mycological flora of Louisiana, particularly the saprobic forms, has not been extensively explored and it therefore seems desirable that some contribution be made toward a systematic treatment of the fungi of this region.

The earliest published report on Louisiana fungi was made by A. Featherman (**1**) in 1871 in the form of a brief list of various species, mostly agarics, collected in Baton Rouge. A single Myxomycete, *Fuligo septica* (as *Aethalium septicum*), was named. Langlois (**2**), in 1878, published a more extensive record of the cryptogamic flora of the state, in which ten Myxomycetes were identified, but among the many subsequent publications on the fungi of this area no further account of them has appeared.

The present paper includes 47 species of Myxomycetes, of which number 42 are reported from Louisiana for the first time. Twenty genera are represented. Almost all the collections were made within a radius of about one mile of the University campus, chiefly between the months of February and August of 1952. As the area of search is extended to other parts of the state, it is to be anticipated that additional species will be encountered.

Collections were found growing on dead wood, unless otherwise indicated. All specimens are deposited in the mycological herbarium of Louisiana State University.

CERATIOMYXALES

Ceratiomyxaceae

Ceratiomyxa fruticulosa (Mull.) Macbr.

Frutifications extensive, branching 3–5 mm tall, covering about one-half meter of a fallen log; spores hyaline, ellipsoid, 10–12 × 6 μ

in diameter. This collection corresponds rather closely with the description given by Lister (3) of the "variety" *flexulosa*. The species is quite variable in morphology and pigmentation, but, as Martin (4) has noted, "the varieties seem unworthy of recognition."

LICEALES

Liceaceae

Tubifera ferruginosa (Batsch) J. F. Gmel.

RETICULARIACEAE

Enteridium Rozeanum (Rost.) Wingate
Lycogala Epidendrum (L.) Fries FIG. 2.
Lycogala exiguum Morgan
Lycogala flavofuscum (Ehrenb.) Rost. FIG. 1.
Reticularia Lycoperdon Bull.

CRIBRARIACEAE

Dictydium cancellatum (Batsch) Macbr.
Lindbladia effusa (Ehrenb.) Rost. FIG. 3.

TRICHIALES

Trichiaceae

Arcyria cinerea (Bull.) Pers. FIG. 9.
Arcyria denudata (L.) Wettst.
Arcyria incarnata (Pers.) Pers.
Arcyria insignis Kalchbr. & Cooke
Arcyria nutans (Bull.) Grev.
Hemitrichia Serpula (Scop.) Rost.
Hemitrichia stipitata (Schw.) Macbr.
Hemitrichia Vesparium (Batsch) Macbr. FIG. 4.
Oligonema flavidum (Peck) Peck FIG. 5.
Oligonema Schweinitzii (Berk.) G. W. Martin
Perichaena vermicularis (Schw.) Rost.
Trichia erecta Rex
Trichia varia (Pers.) Pers.

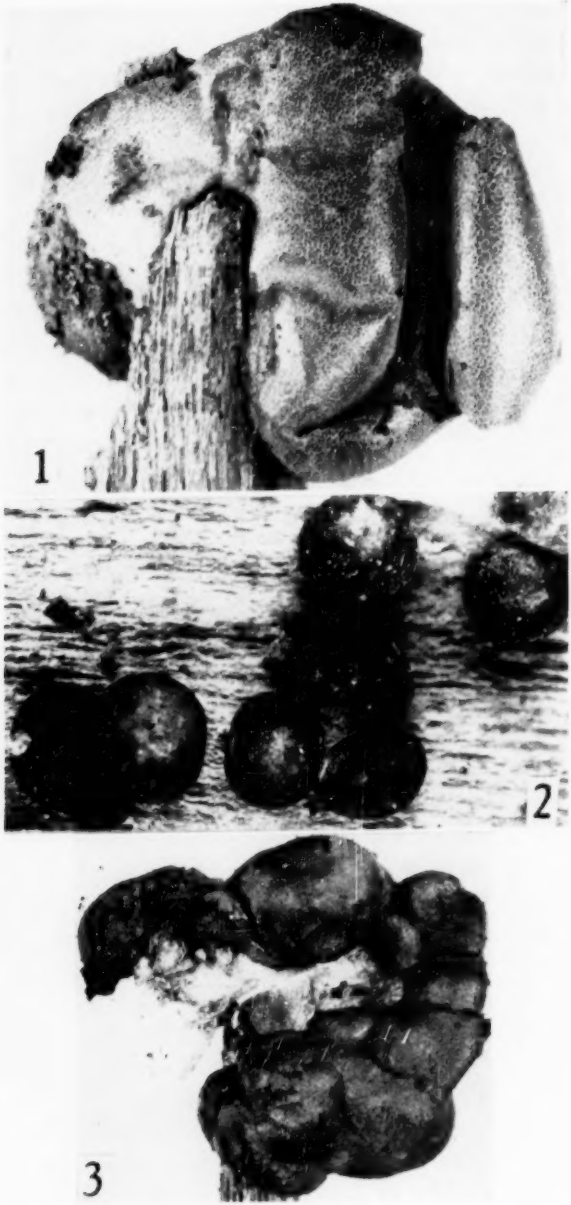
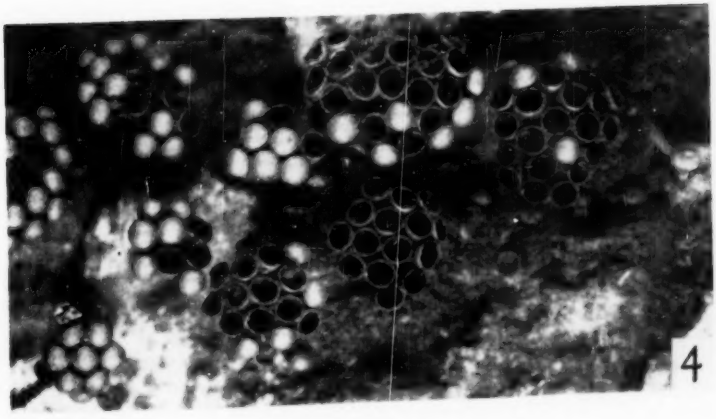


FIG. 1. *Lycogala flavofuscum*, × 2. FIG. 2. *Lycogala Epidendrum*, × 3.
FIG. 3. *Lindbladia effusa*, × 1.



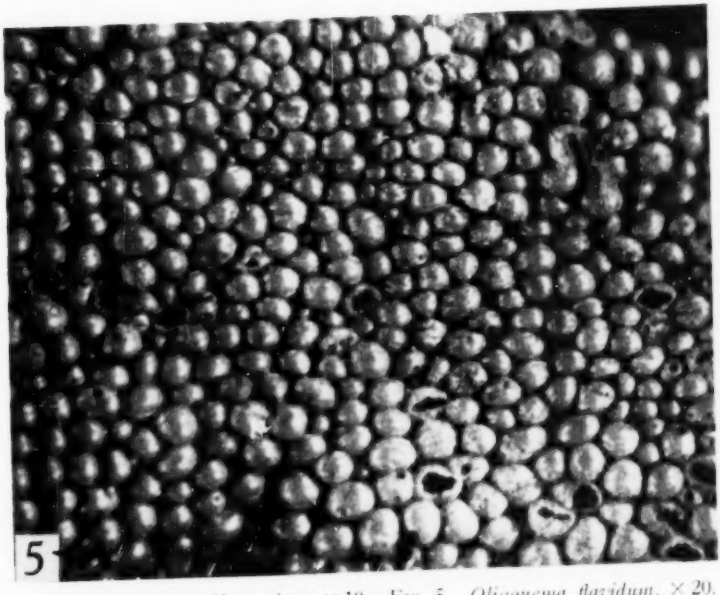


FIG. 4. *Hemitrichia Vesparium*, × 10. FIG. 5. *Oligonema flavidum*, × 20.

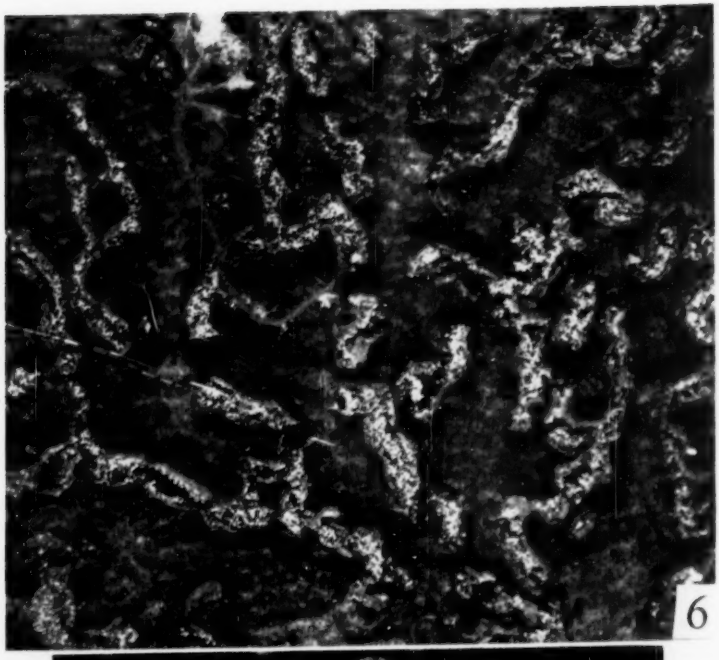
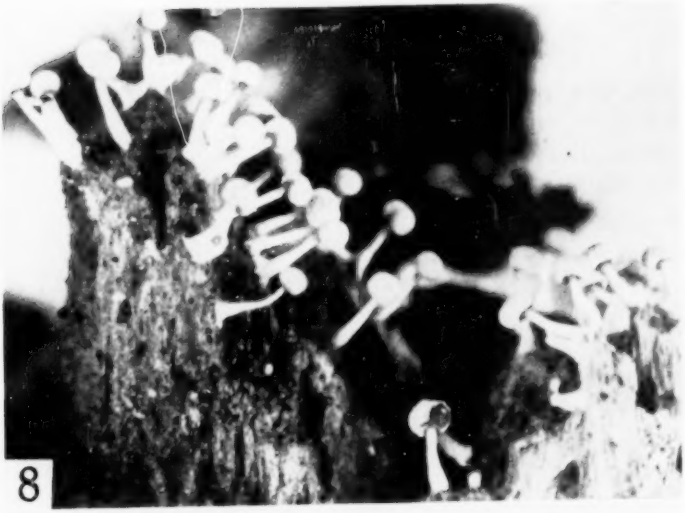


FIG. 6. *Physarum Serpula*, × 5. FIG. 7. *Physarum viride*, × 15.



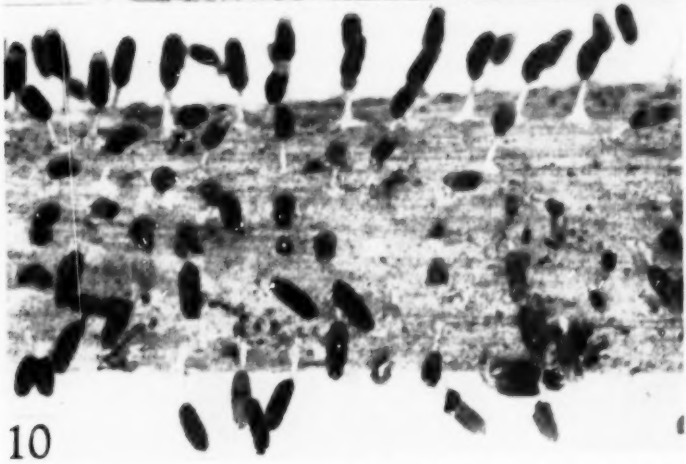


FIG. 8. *Physarum globuliferum*, × 10. FIG. 9. *Arcyria cinerea*, × 10. FIG. 10. *Diachea leucopodia*, × 15.

STEMONITALES

Stemonitaceae

Comatricha longa Peck
Comatricha typhoides (Bull.) Rost.
Diachea leucopodia (Bull.) Rost. FIG. 10.
Stemonitis axifera (Bull.) Macbr.
Stemonitis carolinensis Macbr.
Stemonitis confluens Cooke & Ellis
Stemonitis fusca Roth
Stemonitis hyperopta Meylan
Stemonitis nigrescens Rex
Stemonitis splendens Rost.
Stemonitis virginiensis Rex
Stemonitis Webberi Rex

PHYSARALES

Physaraceae

Badhamia gracilis (Macbr.) Macbr.
Craterium leucocephalum (Pers.) Pers.
Fuligo septica (L.) Weber
Physarella oblonga (Berk. & Curtis) Morgan
Physarum bogoriense Racib.
Physarum cinereum (Batsch) Pers.
Physarum Didermoides (Pers.) Rost.
Physarum globuliferum (Bull.) Pers. FIG. 8.
Physarum nutans Pers.
Physarum polycephalum Schw.
Physarum pusillum (Berk. & Curtis) G. Lister
Physarum Serpula Morgan FIG. 6.
Physarum viride (Bull.) Pers. FIG. 7.

Supplementary note: After the manuscript of this paper had gone to the printer, further collections were made which included nine species not previously reported. It is thought best to include these at this time, increasing the total number of species listed to 56 and the number of genera to 21.

TRICHIALES

Trichiaceae

Arcyria Oerstedtii Rost.
Arcyria occidentalis (Macbr.) G. Lister
Arcyria versicolor Phill.
Hemitrichia abietina (Wigand) G. Lister
Hemitrichia leiocarpa (Cooke) Lister

STEMONITALES

Stemonitaceae

Comatricha lurida Lister

PHYSARALES

Physaraceae

Physarum Bethelii Macbr.
Physarum mutabile (Rost.) G. Lister

Didymiaceae

Didymium Iridis (Ditmar) Fries

The writer wishes to acknowledge the help of Dr. G. W. Martin, who verified the identifications of several of the specimens in this report.

All photographs except that of *Lindbladia effusa* were taken by the author. Thanks are due Dr. E. C. Tims for this print.

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BOTRYOTRICHUM AND COCCOSPORA

MARY H. DOWNING

(WITH 1 FIGURE)

In attempting to identify three of the organisms in the Quartermaster Collection, White and Downing (**1951**) decided that they were conspecific with *Coccospora agricola* Goddard. In the absence of type material, the decision was based on a comparison of these cultures with the description and figures given by Goddard. It was felt that the species *C. agricola* was re-established, although the validity of *Coccospora* Wallr. was questioned at the time. Further investigation reveals that *Coccospora agricola* is a synonym of *Botryotrichum piluliferum* Sacc. and March. and new morphological observations make an emendation of *Botryotrichum* desirable.

The essential characteristics of *Botryotrichum piluliferum* as understood to this time are:

(1) *Spores* hyaline, globose, thick-walled, 10–25 μ in diameter, borne singly or in small clusters.

(2) *Hairs* prominent, rough, olivaceous, borne singly or in tufts.

Coccospora agricola Goddard, as described and illustrated, contains no reference to the sterile hairs. Of the three isolates (QM *336*, *337*, and *991*) described by White and Downing, two possess the sterile hair-like structures. Examination of an authentic herbarium specimen of Marchal's *Botryotrichum piluliferum* (FIG. 1, B, C), grown on agar, reveals prominent, rough, olivaceous hairs. Isolate QM *336* (FIG. 1, D, E, F, G) compares favorably in all respects with this specimen. Blochwitz (**1914**), in a study of *Botryotrichum piluliferum*, gave particular emphasis to the sterile hairs, and demonstrated their origin from the same hyphal base as that from which the spore structures arose. His isolate, from flypaper, has been preserved at the Centraalbureau voor

Schimmelcultures, and it still produces the typical aleuriospores and sterile hairs. This culture is also similar in appearance to QM *336*.

Something which seems to have been overlooked in all of the work reported above is the presence of another spore type. Study of the three cultures in our Quartermaster Collection reveals the presence of hyaline phialides, with phialospores produced in a single chain, which sometimes slimes down into a spherical mass. The phialospores are elliptic and hyaline, with thin smooth walls. The phialides are usually unbranched, short, and borne on the same aerial hyphae which produce the aleuriospores. The structure is most readily detected by direct examination of the petri dish culture under the low power of the compound microscope. Photomicrographs illustrate the two spore types arising from the same hyphae (FIG. 1, D, E, F). First noticed in a plate culture on leaves of *Phragmites communis* on mineral salts agar, the presence of phialides was later confirmed on potato dextrose agar. Within one week after inoculation, phialides begin to develop on the the aerial hyphae, producing rather long chains of spores. The phialospore form was seen in all three isolates, QM *336*, *337*, *991*, and in the Blochwitz culture.

White and Downing (**1951**) believed that the three Quartermaster isolates which they placed in *Coccospora agricola* Goddard have "close natural affinities with the two species upon which Traaen established his genus *Humicola*." Traaen (**1914**) described phialides in *Humicola*. The present discovery of phialides in *Botryotrichum piluliferum* is added support for the close relationship of *Humicola* and *Botryotrichum*. Isolates of both genera have been found to be cellulolytic; both are soil organisms with a preference for alkaline media (Jensen, **1931**; White and Downing, **1951**). The basis for separation now appears to be that *Botryotrichum piluliferum* is for the most part a hyaline counterpart of *Humicola*, having (usually) characteristic sterile hairs.

Since the present consideration reduces *Coccospora agricola* to the synonymy of *Botryotrichum piluliferum*, it seems that a redescription of *Botryotrichum* to include the phialospore form

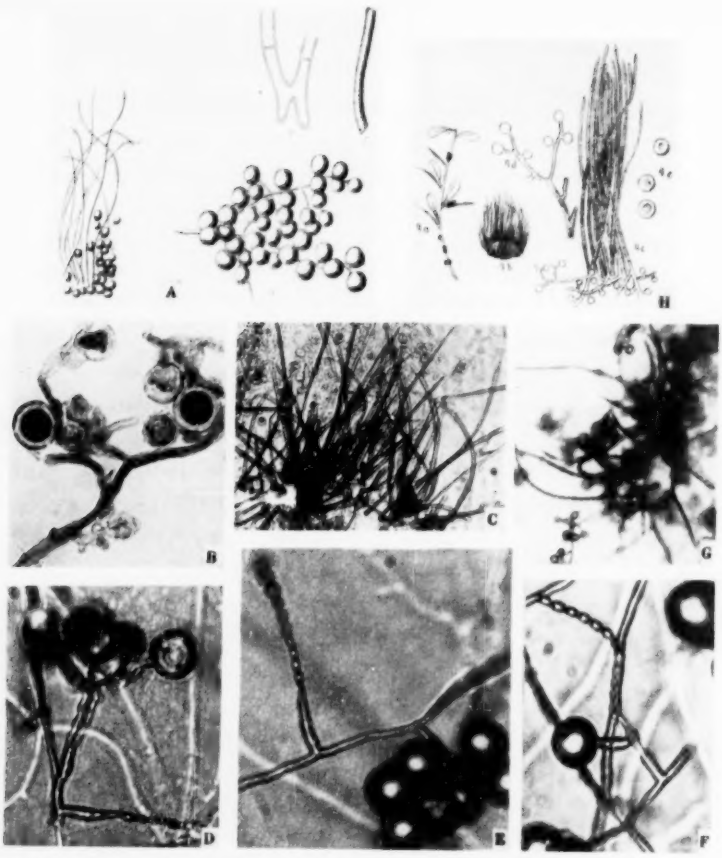

FIG. 1, A–G. *Botryotrichum piluliferum* Sacc. and March. A. Original illustration reduced one-half from Bull. Soc. Roy. Bot. Belg. **24**: 66. B, C. Authentic specimen, Rabenhorst No. *3992*. B. Aleuriospores mounted in 7 percent KOH and stained with phloxine, × 500. C. Sterile hairs and aleuriospores, in Hoyer's mount stained with acid fuchsin, × 100. D, E, F, G. Culture QM *336* grown on a mineral salts agar containing a fragment of corn stalk and leaves of *Phragmites communis*, after 10 days incubation under room conditions; photographed directly from the petri dish surface under a compound microscope; D, E, F, × 550, illustrate the dual spore-bearing hyphae, with aleuriospores and phialospores produced on the same hypha; G, × 130, shows sterile hairs in a tuft, and aleuriospore racemes.

FIG. 1, H. *Botryotrichum* (*Peziotrichum*) *lachnella*, Saccardo's original illustration in Hedwigia (**32**: 56–59) reduced one-half.

should be in order. A modern concept of the species emending the original description follows.

BOTRYOTRICHUM Sacc. and March., Bull. Soc. Roy. Bot. Belg. **24**: 66. 1885. ill. *emend.*

B. PILULIFERUM Sacc. and March., Bull. Soc. Roy. Bot. Belg. **24**: 66. 1885. ill. *emend.*

Coccospora agricola Goddard, Bot. Gaz. **56**: 264–265. 1913. ill.

Fertile hyphae septate, hyaline, very little branched, usually aerial in character, 4–6 μ in diameter, bearing two types of spores. *Aleuriospores* acrogenous, borne either singly on short, undifferentiated side branches of septate hyphae, 12–30 μ long, or in whorls or in racemose clusters of spores in grape-like "bunches"; aleuriospores hyaline, globose, 10–21 μ in diameter, smooth, with walls up to 3.5 μ thick, surrounded by a hyaline membrane which commonly separates from the main wall and ruptures in mounts; Tilleul-buff (Ridgway) in color (in mass). *Phialides* borne at varying intervals, sometimes alternately and sometimes in conjunction with the aleuriospore racemes on the aerial hyphae, hyaline, smooth, septate at the base, usually simple, occasionally branched once or twice at a height of approximately 15 μ, somewhat awl-shaped, measuring approximately 20–22 × 2.8–3.3 μ. *Phialospores* produced in a single chain which sometimes slimes down into a ball-like mass; elliptic, hyaline, thin-walled, smooth, measuring 2.8–4.2 × 1.4–2.8 μ. *Sterile hairs* borne individually or in tufts on the creeping, aerial hyphae; rough, encrusted, olive-grey to brownish, usually simple, septate, measuring 180–240 × 2.8–3.9 μ, widening at the base to 4.2 μ; present in all collections from natural substrata, but not always found in culture.

Type locality: Belgium, on dung.

The colony on potato dextrose agar attains a growth of 2.0 to 4.3 cm in diameter in one week. At first composed of fine white mycelium, it gradually takes on a Tilleul-buff (Ridgway) color, becoming slightly zoned in small concentric areas of heavier and lighter growth, and showing orange color in reverse. The light pinkish-tan color is sometimes entirely covered over by the late appearance of olive-grey sterile hairs.

When the fungus is grown on corn stalks or on the leaves of *Phragmites communis* on mineral salts agar, the sterile hairs appear earlier, tend to grow more in tufts than individually, and quickly give a characteristic grey aspect to the culture; otherwise the growth is similar to that on potato dextrose agar.

Insofar as can be ascertained, *Botryotrichum piluliferum* is not represented in modern reports, but has previously been found on sacking in England (Wakefield, **1919**), on cheese in France (Loubiere, **1924**), and in soil in Canada (Bisby *et al.*, **1933**).

In a discussion of *Botryotrichum*, mention should be made of the species described by Saccardo (**1893**) as *B. lachnella*, which he considered different enough from *B. piluliferum* to place in a separate subgenus, *Peziotrichum*. This name was raised to generic rank by Lindau (**1900**) and has been maintained as a separate genus since. Petch (**1927**), discussing entomogenous fungi collected in Ceylon, presented a critical and detailed account of *Peziotrichum lachnella* and its relationship to *Botryotrichum piluliferum*, and concluded that *Botryotrichum piluliferum* is generically distinct from *Peziotrichum lachnella* because of the dark-colored hyphae of the latter. The similarity between original illustrations of *Botryotrichum* (*Peziotrichum*) *lachnella* (FIG. 1, H) and *Botryotrichum piluliferum* (FIG. 1, A) seems to indicate a close relationship. However, an examination of Saccardo's type of *Botryotrichum lachnella* reveals a growth composed of deep reddish brown, smooth, septate sterile hairs which are occasionally branched and rebranched, and which are always borne at the circumference of a circular, depressed acervulus; they measure 608–1040 × 3.9–5.2 μ. No spores were seen at this time. The growth is confined to small areas and shows no spreading mycelium. Its entire character is quite distinct from that of *B. piluliferum*.

The genus contains only one other species, *Botryotrichum atrogriseum* van Beyma (**1928**), found on fallen wood. A culture of this was deposited at the Centraalbureau voor Schimmelcultures, and has been examined in the present study. It has hyaline to dark mycelium which bears fruiting structures consisting of very dark (black in macroscopic examination), globose,

thick-walled aleuriospores, borne singly at the tip of extremely short, undifferentiated side branches which occasionally show some rebranching. No sterile hairs or phialides were found. On the basis of these characteristics, especially the dematiaceous aspect, this species is excluded from *Botryotrichum* at this time.

Botryotrichum as currently conceived is a monotypic genus, containing only the species *B. piluliferum*.

SUMMARY

Coccospora agricola Goddard is here reduced to synonymy under *Botryotrichum piluliferum* Sacc. and March. The species is shown to produce two types of spores, the predominating aleuriospores and the less prominent phialospores, the latter mentioned here for the first time. On the basis of this dual spore phenomenon, the description of the species is emended. Consideration is given to *Peziotrichum lachnella* Sacc., which had been considered a subgenus of *Botryotrichum;* the two are considered distinct genera in this study. The other species, *B. atrogriseum* van Beyma, is here excluded from the genus because of its dematiaceous character and its lack of sterile hairs and phialides.

ACKNOWLEDGMENTS

I am indebted to the late Dr. Samuel C. Damon for his very kind interest and suggestions in the work under consideration; to the late Dr. W. L. White of the Farlow Herbarium at Harvard University for the loan of authentic material of *Botryotrichum piluliferum* Sacc. and March. (Rabenhorst No. *3992*); and to Prof. Capelletti of Istituto Orto Botanico dell'Università, Padova, Italy, for the type specimen of *Botryotrichum lachnella* Sacc.

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TAXONOMIC STUDIES IN THE HYDNACEAE WITH REFERENCE TO THEIR HYPHAL SYSTEMS[1]

M. A. Ragab

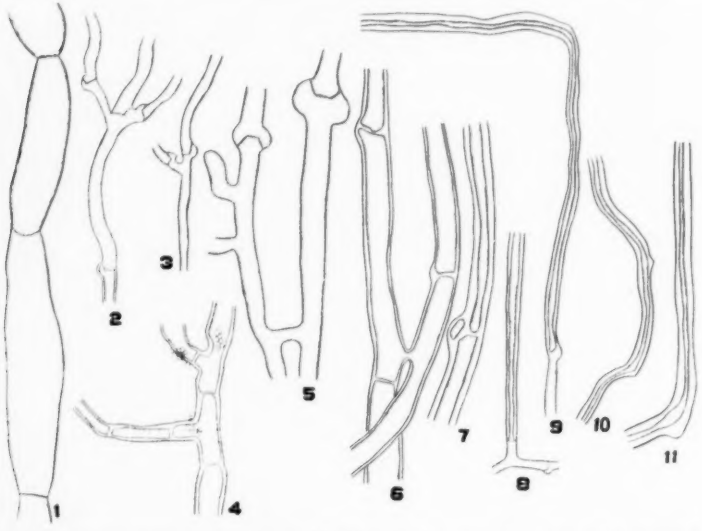
(WITH 11 FIGURES)

In the early systematic arrangement of the higher fungi proposed by Fries, emphasis was given to gross characters which are most easily seen but which are frequently of less value in showing relationship among the groups than other, less conspicuous features. The arrangement based on the shape and appearance of the hymenium, which served Fries, has been subjected to modification and well-based criticism. Fries's system, which emphasized hymenial configuration and which disregarded the more fundamental characters of the basidia stressed recently by Rogers (**10, 11**), and the more detailed morphology of the hyphae pointed out by Corner (**2, 3, 4**) and Cunningham (**5**), has often resulted in unnecessary duplication of names.

Great diversity of opinion exists, not only with regard to the basis of generic limitations in the Hydnaceae but as to the value of various characters which have been used in classification. Emphasis has been placed upon diagnostic characters such as the shape of the fruit body, the character of spines, the presence or absence of sterile bodies such as cystidia and setae, spore color, and consistency of fructification. The consistency characters of the fructification such as tough-fibrous, coriaceous, and fleshy, may be used with various meanings. Toughness may be caused by the presence of skeletal hyphae as in the genera *Gloiodon, Asterodon, Auriscalpium* and most species of *Steccherinum*. The genus *Hydnellum* is characterized by a tough-fibrous structure which is

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany at the State University of Iowa, Iowa City, Iowa, under the direction of Dr. G. W. Martin.

apparently due to moderately thick-walled, not inflated, generative hyphae, rather densely packed and with a tendency to agglutinate into strands in places. The genera *Hydnum* and *Dentinum* have a fleshy texture which may be caused by the thin-



All figures were drawn from KOH-phloxine preparations with camera lucida at × 1680 and reduced in reproduction to × 500.

FIGS. 1–7. Generative hyphae. 1. *Dentinum repandum*, inflated hypha. 2. *Odontia arguta*, profusely branched hyphae. 3. *Oxydontia Himantia*, finely incrusted hyphae. 4. *Odontia laxa*, thick-walled hyphae. 5. *Steccherinum pulcherrimum*, hyphae with double clamp-connections and H-connection. 6. *Hericium Erinaceus*, thick-walled hyphae with clamp-connections and H-connection. 7. *Hericium Erinaceus*, thick-walled hyphae with clamp-connection. FIGS. 8, 9. Skeletal hyphae at their origin from generative hyphae. 8. *Odontia stipata*. 9. *Oxydontia macrodon*. FIGS. 10, 11. Skeletal hyphae. 10. *Gloiodon strigosus*. 11. *Steccherinum ochraceum*.

walled, inflated generative hyphae, loosely united and forming an open tissue.

Any student who has devoted considerable attention to taxonomic study of the Hydnaceae will recognize that little or no attention has been given to the character of the hyphal elements in delimiting genera and species. One object of this study has been to attempt to interpret the structure of the basidiocarp of the

Hydnaceae in the light of the new morphological concepts applied by Corner to the related groups of pore and clavarioid fungi. In the present paper all recognized genera of North America, and species found in Iowa as well as other places have been used for the study. Such a study, supplemented by an examination of gross characters, will furnish data which may be useful in presenting an exact definition of genera and species.

Miller & Boyle (**9**) recognize sixteen genera. The genus *Caldesiella* is treated by them in the Hydnaceae but they pointed out its close relation to *Tomentella*. Rogers (**10, 12**) pointed out that *Caldesiella* and *Tomentella* are probably not separable from each other; the two must be regarded, not as members of two distinct families, Hydnaceae and Thelephoraceae, but as only slightly different aspects of a single genus. Hyphal analysis of *Caldesiella ferruginosa* and several species of *Tomentella* has clearly revealed close affinity between them and Rogers seems justified in his conclusion. The proposal of Rogers to conserve *Tomentella* Pat. against *Caldesiella* Sacc., supported by the Nomenclature Committee of the British Mycological Society (**13**) and by Donk (**6**), was approved by the Stockholm Congress.

Coker and Beers' (**1**) recognition of *Pseudohydnum* Rick is unfortunate. It should be noted that the generic name *Pseudohydnum* Rick 1904 is antedated by *Pseudohydnum* Karsten 1868. There is no doubt that *Pseudohydnum* Rick is a later homonym of *Pseudohydnum* Karst. in the Tremellaceae, and must be replaced by *Hydnodon* Banker 1913. Martin (**7, 8**) revived Karsten's genus and is using it in place of the later *Tremellodon*.

As a result of this study it has been found that the majority of the species examined are monomitic. The genera *Radulum*, *Grandinia*, *Hydnum*, *Hydnellum*, *Hydnodon*, *Dentinum*, and *Hericium* are monomitic. The monotypic genera *Asterodon*, *Auriscalpium*, and *Gloiodon* are dimitic. The species of *Steccherinum*, *Oxydontia*, and *Mucronella* may be either monomitic or dimitic. The majority of the species of the genus *Odontia* are monomitic; of twenty species examined only three have a dimitic system of hyphae. On the other hand, of eight species of *Steccherinum* only one is monomitic. In *Oxydontia*, of six species

studied, only one is dimitic. In *Mucronella*, of three species studied, only one is dimitic.

It is apparent that some of these genera will probably have to be divided, since it is doubtful whether both monomitic and dimitic species should be included in *Steccherinum*, *Odontia*, *Oxydontia*, and *Mucronella*. Corner (**4**) has already transferred dimitic species of *Mucronella* to *Deflexula*. However, it seems wiser at the present time to expand the concept of these genera so as to include these species, at least until tropical specimens are studied.

Two hyphal systems are present in the family, monomitic, and dimitic. They may be composed of two hyphal types generative and skeletal. The terms monomitic, dimitic, generative and skeletal are used in the sense of Corner.

In the recognition of genera, a complex of characters rather than a single character is considered. The writer's present knowledge of the Hydnaceae seems to warrant the recognition of fourteen genera. The genus *Hydnellum* is emended so as to include the species formerly included in the genus *Phellodon*. Both *Hydnum albonigrum* Peck and *Hydnum amicum* Quél. agree in hyphal structure with *Hydnellum velutinum* (Fries) Karst. The major differences are in spore color and markings and in superficial external characters, none of which are regarded as sufficient to justify generic segregation. Both have been referred to both *Phellodon* and *Calodon*, neither genus, however, valid against *Hydnellum* as here emended. It is necessary, therefore, to propose as new combinations **Hydnellum alboniger** (Peck) comb. nov. and **Hydnellum amicum** (Quél.) comb. nov.

KEY TO THE GENERA OF THE HYDNACEAE

a. Subiculum lacking or represented by a fugacious mycelium; spines simple or branched, arising directly from substratum; monomitic or dimitic; on wood and bark.....I. *Mucronella*
a. Subiculum present; trama dark or pale.....b
b. Fructification resupinate, reflexed to pileate and laterally sessile, or stipitate; monomitic or dimitic; on wood, or cones of conifers....c
b. Fructification pileate and stipitate; monomitic; on the ground.....k
c. Trama dark; dimitic.....d
c. Trama pale; monomitic or dimitic.....e
d. Resupinate, floccose; stellate setae present; clamp-connections lacking, spores hyaline, smooth; on wood.....II. *Asterodon*

d. Resupinate or reflexed to pileate and laterally sessile; spines borne on tough profusely branched processes partially submerged in a brownish tomentum; clamp-connections present; spores hyaline, faintly roughened; on wood.....III. *Gloiodon*

e. Resupinate, thin; floccose, crustaceous, ceraceous, or subcoriaceous.....f

e. Reflexed, sessile or pileate and stipitate, rarely resupinate; fleshy, tough, to coriaceous.....i

f. Cystidia present; texture variable; monomitic or dimitic; clamp-connections present or absent; skeletal hyphae unbranched; spores smooth or echinulate; on wood.....IV. *Odontia*

f. Cystidia absent.....g

g. Ceraceous; teeth blunt, often deformed and irregularly scattered; rarely reflexed; monomitic; on wood.....V. *Radulum*

g. Texture variable; strictly resupinate; teeth hemispherical to cylindrical or subulate.....h

h. Teeth in the form of short, fragile, hemispherical or cylindrical warts; monomitic; on wood.....VI. *Grandinia*

h. Teeth or spines typically long, conspicuous, slender, usually terete; monomitic or dimitic; on wood.....VII. *Oxydontia*

i. Fructification abundantly branched or pulvinate, fleshy-fibrous; monomitic; generative hyphae often very thick-walled; gloeocystidia usually present; on wood.....VIII. *Hericium*

i. Fructification not branched nor pulvinate; subfleshy to coriaceous; dimitic, rarely monomitic; cystidia present or absent.....j

j. Fructification laterally stipitate, stipe long, slender; pileus coriaceous; dimitic; cystidia present; spores hyaline, minutely roughened; on pine cones.....IX. *Auriscalpium*

j. Fructification reflexed to laterally stipitate (rarely resupinate), subfleshy to coriaceous; often dimitic, rarely monomitic; cystidia present or absent; spores hyaline, smooth; on wood..X. *Steccherinum*

k. Fructification fleshy, fragile; hyphae inflated, thin-walled; spores hyaline, smooth.....XI. *Dentinum*

k. Fructification not fragile; spores subhyaline to distinctly colored, echinulate or tuberculate.....l

l. Subgelatinous; hyphae thin-walled, not inflated; spores coral pink, tuberculate.....XII. *Hydnodon*

l. Fleshy-tough to fibrous.....m

m. Fleshy; hyphae thin-walled, inflated, clamp-connections present or absent; spores brown, tuberculate.....XIII. *Hydnum*

m. Fibrous-tough; hyphae rather thick-walled, not inflated; clamp-connections not seen; spores either brown and tuberculate or subhyaline and echinulate.....XIV. *Hydnellum*

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ABORTIVE "PERITHECIAL" PRODUCTION BY PHIALOPHORA VERRUCOSA

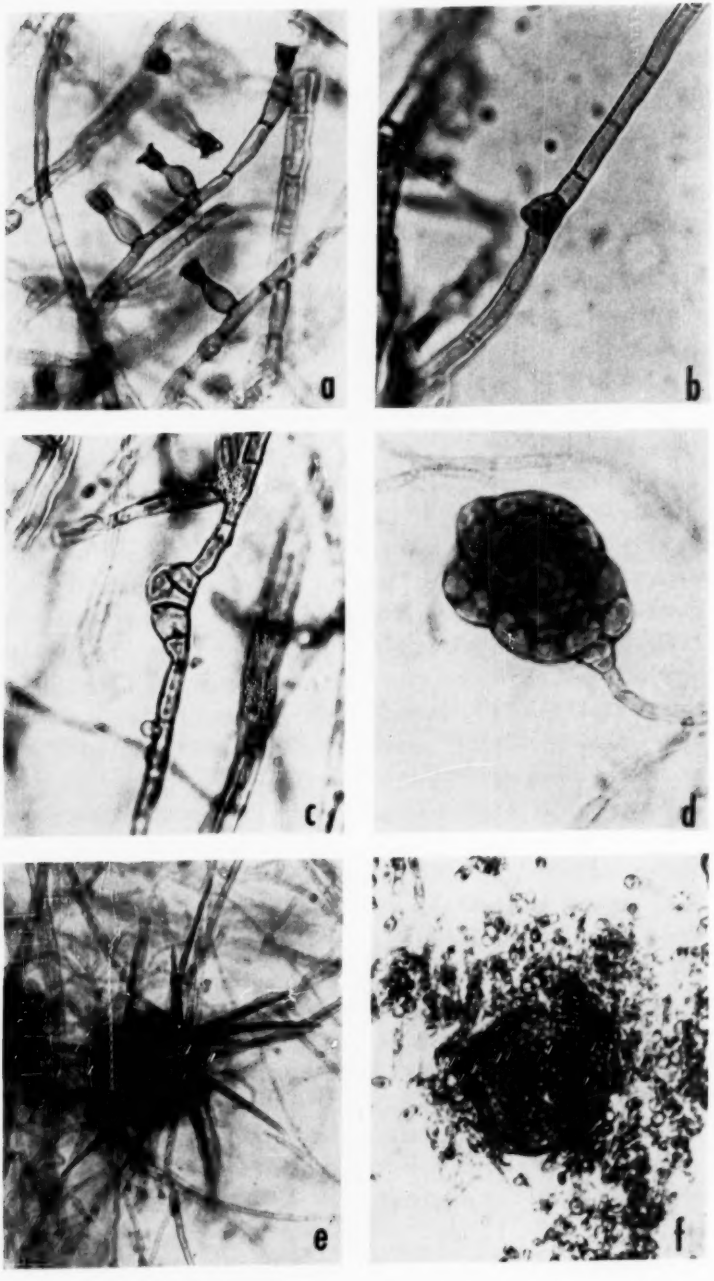
LIBERO AJELLO AND LALIAH RUNYON

(WITH 2 FIGURES)

Reproduction in the majority of the fungi pathogenic to man and lower animals is by asexual means. Only two species, *Allescheria boydii* Shear, 1922 (**7**), and *Piedraia hortai* (Brumpt) Fonseca & Area Leao, 1928 (**3**), are known to possess a perfect stage. Both are classified among the ascomycetes.

Despite the rarity of sexual reproduction among fungi causing animal diseases, it is possible that under certain natural or experimental conditions any or all of these imperfect organisms may become capable of producing sexual spores. Indeed such a phenomenon was observed by Emmons (**2**) to have occurred spontaneously in an isolate of *Monosporium apiospermum* Saccardo, 1911 (**5, 6**), long maintained in culture. This organism produced perithecia and ascospores referable to *A. boydii*. A similar event was thought to have occurred in our laboratory when a student drew attention to some unusual structures resembling perithecia in a teased mount of *Phialophora verrucosa*.

P. verrucosa is a well-known agent of chromoblastomycosis among humans (**1**) and is one of the fungi responsible for the blueing of timber (**4**). Reproduction in this "fungus imperfectus" is by means of unicellular spores, borne semi-endogenously on flask-shaped conidiophores (FIG. 1a). The observation of large dark spherical bodies bearing setae (FIG. 1e) in a large series of cultures of one stock strain was thought to indicate another means of reproduction. However, despite their morphologic similarity to perithecia, these structures have all proven to be infertile. Repeated attempts to cross this isolate with several other strains of this fungus maintained in the laboratory, and the use of various types of natural and semi-synthetic substrates to induce the complete development of the "perithecia" have all been fruitless.


a
b
c
d
e
f

Despite this failure, it has been thought worth while to describe briefly the development of what appears to be abortive perithecia in *P. verrucosa*.[1] This is done with the hope that other observers may be stimulated to search for similar developments in their cultures of this organism and perhaps observe or bring about completion of the cycle.

The "perithecial" initials arose in an intercalary or terminal position in the mycelium (FIG. 1 b, d). By multiplication of the primary cell, a solid, multicellular, more or less spherical body

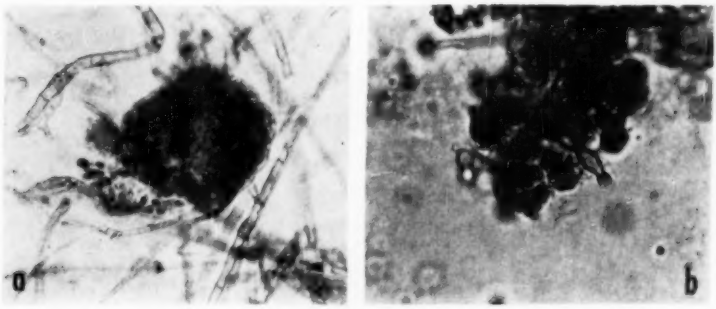


FIG. 2. a. Crushed "perithecium" showing escaping contents, ×160; b. Group of pseudoparenchymatous cells from interior of a "perithecium" (aceto-carmine mount), ×160.

was developed (FIG. 1, d). As this structure increased in size dematiaceous filaments were formed on its surface (FIG. 1 e). These setae were attenuated at the tip and were composed of one or several cells. They measured from 20–60 μ in length and averaged 4 μ in diameter. The "perithecia" at their maximum size were composed of a mass of pseudoparenchymatous cells surrounded by a peridium, one cell layer thick (FIG. 1 f), and ranged from 20–90 μ in diameter.

[1] This particular isolate of *P. verrucosa* was received from Tulane University, but unfortunately a history of its ultimate source could not be furnished.

FIG. 1. a. Conidiophores of *P. verrucosa*, ×490; b. Intercalary development of "perithecial" initial, ×694; c. Early stage of cellular multiplication, ×490; d. Multi-celled terminal "perithecial" initial, ×490; e. "Perithecium" with setae, ×190; f. Cross section of fixed and stained "perithecium" showing peridium and undifferentiated interior, ×245.

No trace of ascogonial initials were observed in fixed and stained preparations. When pressure was applied to the perithecia, the peridium ruptured readily permitting groups of pseudoparenchymatous cells to escape (FIG. 2 a, b).

Several other isolates of *P. verrucosa* were studied along with the Tulane culture but none produced these interesting structures.

To date, this is the extent of our observations. However, nutritional and physiological studies currently are under way to determine whether the conditions necessary to complete this apparent attempt at sexual reproduction can be discovered.

The authors are indebted to Mr. John P. Gust of the Audio-Visual Services Section for the photomicrographs.

SUMMARY

1. In a culture of *Phialophora verrucosa*, the development of perithecial-like structures was observed.

2. Attempts to induce completion of their development ended in failure.

3. A description of the developmental states of these bodies is presented.

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HUMICOLA GRISEA, A SOIL-INHABITING, CELLULOLYTIC HYPHOMYCETE

W. Lawrence White[1] and Mary H. Downing[2]

This is the second paper[3] of a proposed series dealing with a group of Hyphomycetous soil fungi characterized morphologically by the copious production of globose, thick-walled aleuriospores as the principal spore type, and physiologically by the fact that they are in the main among the strongest of the cellulose-decomposing fungi. The present installment is confined to a consideration of *Humicola grisea*.

HISTORICAL AND TAXONOMIC SIGNIFICANCE OF THE SPECIES

The genus *Humicola* was established by Traaen (**1914**) on two species which he isolated repeatedly from Norwegian soils. His first and most frequently isolated species, *H. fuscoatra*, was characterized by spores on the order of 6–9–12 μ in diameter with walls of medium thickness and of a very dilute color; his second, *H. grisea*, by larger spores, 12–17 μ in diameter, with thicker and much more deeply colored walls. A modern investigator examining the respective type cultures, which have been maintained at the Centraalbureau since about 1912, might raise a question as to whether or not they merely represent extremes of variation within a single species. However, an adequate number of cultures has now been accumulated to demonstrate beyond all doubt that there actually are two distinct species as indicated by Traaen. The first species of Traaen, i.e., *H. fuscoatra*, is to be regarded as the generic type.

Traaen's *H. grisea* took on a peculiar and interesting significance taxonomically when Mason (**1933**, p. 56) speculated that it

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[2] Pioneering Research Laboratories, U. S. Army Quartermaster Corps, Philadelphia, Pa.

[3] The first was published in *Mycologia* **43**: 645–657. 1951.

(under the name *Monotospora Dalcae* Mason) was synonymous with *Monotospora toruloides* Corda, the single species upon which Corda (**1837**) had founded the genus *Monotospora*. This being true, it would of course follow that *Humicola* Traaen was a synonym of *Monotospora* Corda. Actually, Mason, a few years later (**1941**, p. 113) discovered the paper of Traaen and expressed the opinion that Traaen's *Humicola* should be referred to synonymy under *Monotospora* Corda but not under what he called "*Monotospora* Sacc." Some years after the work of Corda, Saccardo (**1886**) had taken up the generic name *Monotospora* Corda in a sense that excluded Corda's *Monotospora toruloides*. Various writers have commented upon the taxonomic complications involved. Various ways have been suggested for dealing with the nomenclatural problem. That which has been basic is Mason's proposal to refer *Humicola* Traaen to synonymy under *Monotospora* Corda and to use "*Monotospora* Sacc." for the several species, probably of a miscellaneous nature, which Saccardo transferred to his (Saccardo's) *Monotospora* Corda. What Mason calls "*Monotospora* Sacc." would then, according to Mason, be conserved against *Monotospora* Corda leaving the way open for the acceptance of *Humicola* as a good genus.

Diehl (**1949**, p. 279) summed up Mason's point of view, and also his own, in the following words: "He [Mason] pointed out that, if *Monotospora* of Saccardo should be conserved against Corda's prior name, *Humicola* as a synonym of the discarded *Monotospora* of Corda would be legitimate with *H. fuscoatra* Traaen as the type species. Because of its convenience this should be a welcome taxonomic disposition of that hitherto debated form-genus"

The present paper deals with *Humicola*, and no detailed analysis of the early history of *Monotospora* will be attempted; nor would it be advisable on our part until we had gained a better knowledge than we now have of the species placed in *Monotospora* by Saccardo. Brief comment appears desirable, however, in order to explain our acceptance of *Humicola*. Rogers (**1949**, p. 459; **1950**, p. 28) has, in our opinion, dealt effectively with "*Monotospora* Sacc." in stating that there simply is no such thing. Must *Humicola*

then be relegated to synonymy under *Monotospora* Corda? If Mason's contention that *Humicola grisea* is synonymous with *Monotospora toruloides* Corda is correct, the answer obviously is 'yes.' We prefer, however, to move in what appears to be a current drift toward the acceptance of *Humicola* and we believe there are reasons for doing so that are more basic than any heretofore suggested.

There is no overwhelming evidence that Corda's *Monotospora toruloides* is the same as Traaen's *Humicola grisea*. We submit that Corda's drawing could just as well represent *Papularia sphaerosperma* (Pers.) Höhn. Corda's organism formed a black effuse growth on a dead monocotyledonous substratum. This is the characteristic substratum and habit of growth for the *Papularia*, but not for *Humicola grisea*. The latter, insofar as has been demonstrated, is strictly a soil species or an inhabitant of humus or decaying wood in the soil. It has never been found under natural conditions in the form of a sporulating, hyphomycetous turf on the surface of any plant debris.

The microscopic morphology depicted by Corda fits the *Papularia* at least as well as the *Humicola*. The proportionate thickness of wall to size of spore by Corda is approximately correct for that of the lenticular spores of *Papularia* as they are commonly seen in face view in microscope mounts and is too great for *Humicola*. There is evidence of the lenticular shape in at least one of the spores drawn by Corda.

At any rate the two species with which the writers are principally involved, *grisea* and *fuscoatra*, are now in *Humicola*. Neither of them has a legitimate combination in *Monotospora*. Transferring them to *Monotospora* would require changes in nomenclature and add to synonymy. The "burden of proof" should fall upon the individual who makes the change. In this case we can offer neither proof of necessity for such transfers nor evidence of desirability from the point of view of phylogeny or nomenclatural stability.

Practically all the records, or possible records, of this species have come from studies in soil microbiology. It is of interest to note that of the many cultures of *Humicola* that originated from

industrial and military materials in the tropical deterioration program all were *H. fuscoatra*. This is in accord with the findings of Diehl (**1949**). The present writers believe that they have seen all of these cultures regardless of the laboratory in which the isolations were made.

The earliest acceptable records of *H. grisea* are those of Jensen (**1912**) and Dale (**1912**). This was just prior to Traaen's establishing the genus *Humicola*. Jensen's isolates were wrongly assumed by him to be identical with an organism that had been described by Morgan in 1895 as *Monotospora nigra* Morgan, and Dale's was wrongly presented (Dale **1912**; Smith and Ramsbottom **1912**) as *Basisporium gallarum* Moll.

Mason is to be credited with the sorting out of the cultures involved in the early history of *Humicola grisea*.

SYNONYMY

HUMICOLA GRISEA Traaen, Nyt. Mag. Nat. **52**: 34. 1914.

Monotospora Dalcae Mason, Annotated account of fungi received at the Imperial Mycological Institute, List II (Fascicle 2), p. 50, 1933.

Melanogone puccinioides Wollenw. and Richter, Zentralbl. fur Bakt. Abt. 2. **90**: 76. 1934.

GEOGRAPHIC DISTRIBUTION, HABITAT, MATERIAL EXAMINED

Massachusetts: Harvard Forest *I–1–2*, *I–1–17b*, and *I–6–2*, isol. at Harvard Unit., 12 Oct. 1949 by Frank L. Raymond from well-drained sandy loam at point 4 inches below surface of mineral soil in 26 yr. old pine-spruce plantation, Harvard Forest, Petersham, Mass., Plot 26N., Prospect Hill Compartment I; Harvard Forest *II–1–20*, *II–7–9*, and *II–7–10*, isol. at Harvard Forest, 6 July 1950 by Frank L. Raymond from loam soil taken at line of demarcation between organic matter and mineral soil in same plot as above.

New York: Said by Jensen (**1912**) to be common in cultivated soil at Ithaca and isol. commonly in summer, winter, and fall. Jensen made the combination *Mycogone nigra* (Morgan) Jensen

on the erroneous assumption that he was dealing with *Monotospora nigra* Morgan. The latter (type specimen loaned us by Dr. G. W. Martin) is a very different thing. Jensen's description is almost certainly based on *Humicola grisea* but the specimen he cites is lost (Mason, **1933**, p. 54; also personal communication from G. C. Kent, June 1951). Whether or not all of Jensen's isolates should be referred to *H. grisea* is, of course, unproven.

New Jersey: Waksman (**1916**) reported an isolate which he questionably labeled *Basisporium gallarum* Moll. from iron (48 per cent) soil taken at a depth of 8–10 in. in a peach orchard at Keyport. This would appear from his description to be *H. grisea*. It did not appear in several other soils. The isolate proved to be strongly active in decomposing a chemically modified cellulose. Waksman mentioned isolates of *Humicola* sp. in several additional papers but their specific identity is unknown. The writers have seen only one isolate of *Humicola* by Waksman, his No. *63*, which is *H. fuscoatra* as indicated by Mason and Diehl. No data are available concerning its origin (personal communication from Waksman).

Manitoba: ATCC *6725* (QM *512*), a culture sent (as *Monotospora Daleae*) by Dr. J. E. Machacek, then of the Dominion Laboratory of Plant Pathology, Winnipeg, to the Am. Type Cult. Col. in 1938 without detailed data. This is undoubtedly confirmatory of the reports by Bisby, James and Timonin **1933**, p. *266*; Bisby, Buller and Dearness **1933**, p. 100; Bisby **1938**, p. 121, that the species is common in soils of grasslands and wheatfields in Manitoba "but not found in other soils."

Saskatchewan: Isolated from roots of wheat from Indian Head (Bisby **1938**, p. 121).

Finland: Based on the work of Vartiovaara (**1935**).

Poland: Based on work of Felsz-Karnicka (**1935**). Cultures not seen by us.

Norway: Culture obtained from C.B.S. and accessioned here as QM *993*, isolated from soil in Norway by Traaen (see Traaen **1914**), regarded as the type of the species. Traaen, using filter paper as an isolation medium, obtained cultures of the species from 17 samples of substrate from such habitats as decaying tree

trunks, at least some of which were coniferous, bits of leaves from various hardwoods, and samples of soil from near surface of meadow, muck, an anthill, graveyard, brook bed and potato field. It was especially common in field and meadow soil and rotten tree trunks.

Germany: Based on work of Wollenweber and Richter (**1934**). Organism made by them the basis of a new genus and species, *Melanogone puccinioides,* and later properly referred to *Humicola* by Mason (**1941**). Said to be an inhabitant of forest litter, decaying tree roots, and decaying structural timber at or just below ground level, specifically in roots of *Ulmus scabra-montana* killed by Dutch elm disease, forest litter of birch-pine and of spruce; not forming any conspicuous surface deposition of mold growth. A culture deposited at C.B.S. by Wollenweber was examined by us.

England: Culture obtained from C.B.S. and accessioned here as QM *992,* isolated by Dale from soil at Woburn, England, the soil having been "continuously manured for 38 years with sulphate of ammonia" and having acquired a "distinctly acid reaction." (See Dale **1912**, Smith and Ramsbottom **1912**, Mason **1933**, **1941**, Wakefield and Bisby **1941**.)

Ceylon: Culture cited by Mason (**1933**), from 'Adco,' C. H. Gadd *87*. Not examined by us.

Australia: Culture cited by Mason (**1933**), from soil, Melbourne, E. McLennan. Not examined by us.

CHARACTERISTICS OF THE SPECIES

Morphological. Humicola grisea is known only in pure culture, there being as yet no authentic record of its ever having been identified in the form of a superficial fruiting layer on natural substrata, which is characteristic of so many of the Dematiaceae. A possible exception is the fact that Wollenweber obtained aleuriospores and phialospores on naturally infected wood which he removed from its source and exposed to another set of conditions. On agar media growth is fairly rapid and aleuriospores are formed among the underlying aerial hyphae, quickly, and usually in some abundance. FIG. 1 shows duplicate plates of four isolates grown in parallel series under room conditions on potato dextrose agar

(per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes). It will be noted that the heavy, fairly compact, cottony mat in eleven days nearly reached the margin of the 9 cm petri dishes.

The four strains shown in FIG. 1 were used for comparison of colony characteristic mainly because they were the four most conveniently available at the time. Incidentally, they represent wide variation in age of isolate and geographic range. (A) represents a culture isolated from soil in England at least 38 years ago; (B) was isolated from soil in Norway at least 36 years ago; (C) was obtained from soil at Petersham, Massachusetts 1 year before the photo was taken; (D) was taken from a Manitoba soil about 20 years ago. These strains exhibit what most workers would consider to be a remarkable stability in culture and an equally remarkable uniformity among strains of a species. The four strains show no differences that could be put into words with the exception of (A) where the colonies exhibit a dark central area, due largely, if not entirely, to the formation of globules of liquid on and within the hyphal mat.

Growth is at first nearly white, then a very pale gray, gradually deepening and with age reaching a fairly dark gray. The color is mostly in the dark aleuriospores which develop among the hyphae. The pedicels are also noticeably dark as seen under the microscope, while the hyphae, though hyaline as seen individually, may contain a certain amount of pigment as seen in mass.

The aleuriospores (FIG. 2, A–D, and F–J) are smooth, dark, single-celled, typically globose, with an occasional one varying to obovoid or pyriform. The walls are of substantial thickness. The spores fundamentally are borne singly but it is not unusual to find two (FIG. 2, A, F) or three clinging together. Certain writers have been led to make reference to two-celled spores through the interpretation of an inflated hyphal cell adjacent to the spore (FIG. 2, G left of center, F, and I) as part of the spore. Most of the spores fall within the range of 12 to 15 μ in diameter with any strain exhibiting an occasional spore under and over this range. Detailed measurements have been made for five strains with the following averages for 70 spores:

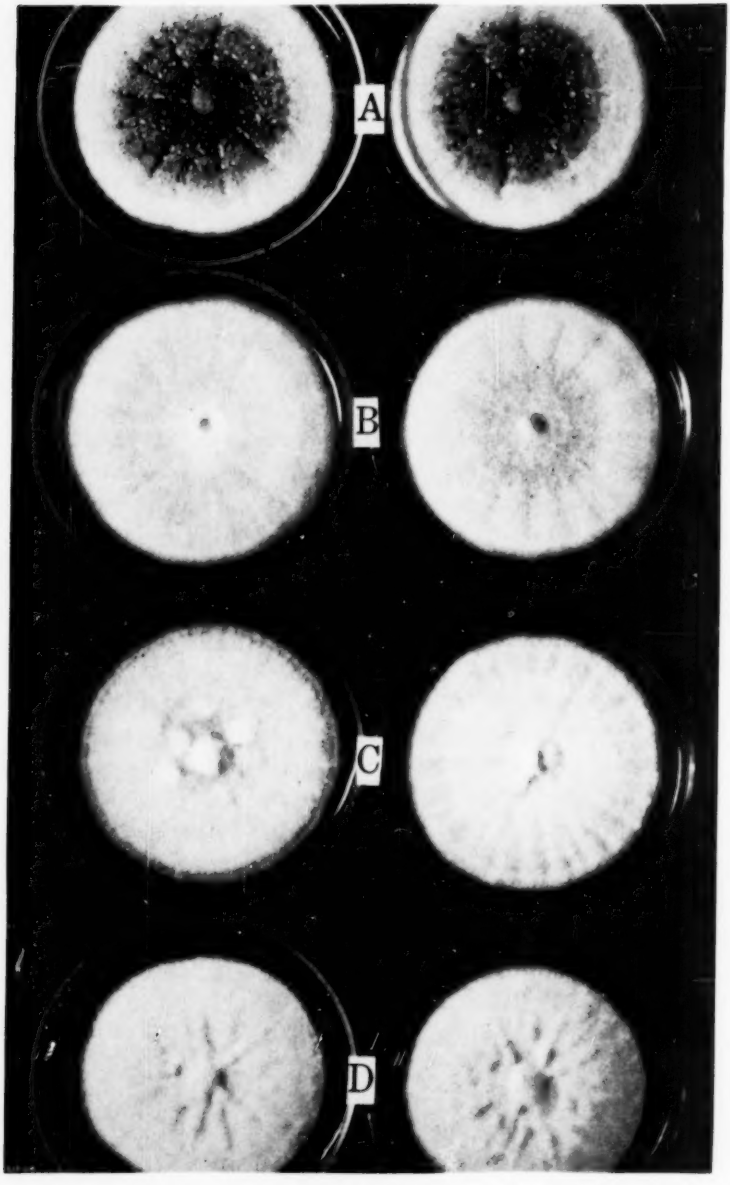

FIG. 1. Four cultures of *Humicola grisea* grown in parallel series for comparison of gross colony characteristics: A. QM 992, the culture iso-

1. QM *993*, Traaen's type from 6-weeks-old colony on potato dextrose (*2* percent dext.) agar, mounted in water = 13.2 μ.

2. QM*992*, Dale's culture, grown in parallel series with the preceding, also mounted in water = 13.2 μ.

3. Harvard Forest *1–1–2*, in parallel series with the two preceding = 14 μ

4. Harvard Forest *11–1–20*, from a potato dextrose agar (*2* percent dext.) stock slant of unknown age, probably from refrigerated storage = 12.8 μ.

5. ATCC *6725* (QM *512*), grown in parallel with 1–3 above = 12.4 μ.

These measurements agree closely with those recorded by Traaen, Bisby, and others, and are recorded only for the purpose of showing that there is no great amount of variation among strains.

The variations in microscopic characters described above are variations within a strain. No noteworthy variation has been found among strains in the course of routine examinations and comparison of photomicrographs. In accompanying plates the spores of the recent isolate Harvard Forest *1–1–2* (FIG. 2, G–J) may be compared with those of Traaen's type culture (FIG. 2, A–C).

Traaen, Wollenweber, and Mason all found phialospores and phialides in their cultures in addition to the aleuriospores. These were inconspicuous, however, and were encountered only under certain conditions. Traaen noted their development as a whitish covering on old colonies grown on filter paper and mineral salts. Mason found them in all the strains with which he worked, but only on potato dextrose agar, and in all but one isolate they were always in old cultures after the period of active aleuriospore formation. Wollenweber produced them on the surface of naturally

lated by Dale prior to 1912 from soil in England; B. QM *993*, Traaen's type culture isolated from Norwegian soil prior to 1914; C. Harvard Forest *1–1–2* from soil at Harvard Forest, Petersham, Mass. in 1949; D. ATCC *6725* isolated from soil in Manitoba about 1930. Colonies started by transplanting from stock cultures a small piece of agar with fungus. Grown on potato dextrose agar (per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes), 35 cc medium per dish, room conditions, 11 days. Magnif. approx. ½. Photo by Frank White.

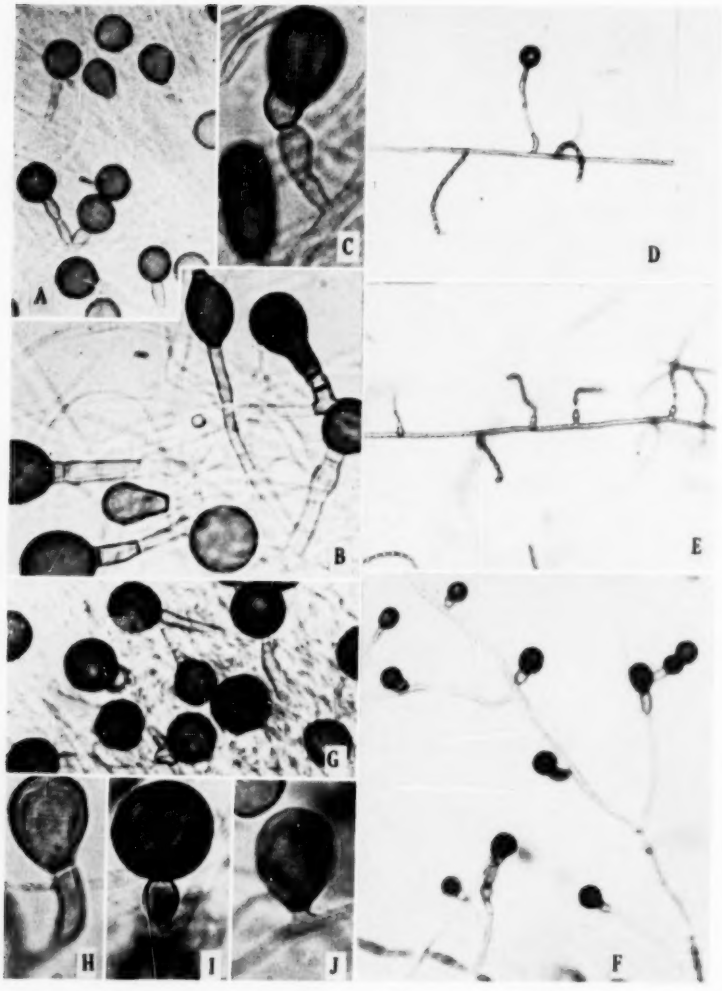

FIG. 2. Photomicrographs of *Humicola grisea*. A–F. QM *993* from plate cultures of Traaen's type: A, × 500, B, × 775, and C, × 1000, aleuriospores of the culture on peptone-glucose (0.5–1 percent) agar after 12 days under room conditions, mounted in lacto-phenol, unstained; A and B from growing culture, photomicrograph by Leo Kaplan; C from the same culture after it had been dried down as an herbarium specimen, photomicrograph by Paul Brown. D, E, F, all × *260*. Photomicrographs by the junior author, taken directly from a culture on mineral salts agar containing fragments of corn stalk and leaves of *Phragmites communis* after 12 days at

infested wood, possibly by placing the pieces in a moist chamber. The phialides appear as single, elongate, tapering cells about 8–16 × 2–3 μ, seated laterally on a hypha. They extrude small, hyaline, obovoid spores about 3 × 2 μ, in chains or in balls. In the present study phialides have again been demonstrated in QM *993*, a culture from Traaen's type grown on fragments of corn stalk and on leaves of *Phragmites communis* in mineral salt agar (30° C, 12 days) (FIG. 2, D, E). Under the same conditions, no phialides were found in the six other cultures examined.

Physiological. The species appears to be characterized by a physiological stability comparable to the morphological. This at least is true of its cellulolytic ability. In a previous paper (see footnote, first page), a table was presented comparing the cellulolytic activity of numerous isolates of four allied species (*Humicola grisea, H. fuscoatra, Coccospora agricola,* and *Monotospora lanuginosa*). With the exception of *M. lanuginosa*, all exhibited strong cellulolytic properties.

The cellulolytic activity of these isolates did not appear to be affected by the length of time that they had been maintained on artificial media containing a sugar as the carbon source. The minimum periods under which the isolates under test were kept on such media:

	QM *992*—38 years
	QM *993*—36 years
ATCC *6725* =	QM *542*—20 years
Harvard Forest *1–1–2* =	QM *994*— 1 year
Harvard Forest *1–6–2* =	QM *995*— 1 year
Harvard Forest *1–1–17b* =	QM *996*— 1 year

30° C, no mount; D shows aleuriospore and phialospores produced from same basal hypha; E illustrates phialospore production, and F shows branching hyphae bearing aleuriospores. G–J. Photomicrographs by Paul Brown of aleuriospores from Harvard Forest *1–1–2* culture: G, × 500. From potato dextrose agar mounted in lacto-phenol, unstained; H, × 1000. Spore on an elongate, cylindric hyphal branch from an unknown medium, mounted in lacto-phenol, stained with fast green; I, × 1000. Spore seated on an inflated hyphal cell, mounted in lacto-phenol, stained with cotton blue; J, × 1000. Spore originating directly from the side of a hypha, mounted in lacto-phenol, stained with fast green.

In an attempt to round out the present study, excerpts from Traaen's work (**1914**) are presented covering the conditions for growth:

Temperature: 5–30° C; optimum 20–25° C.

Carbon Sources: Good: Glucose, sucrose, fructose, inulin, starch, xylan, pectin. Poor: Glycerol, maltose, mannitol.

Nitrogen Sources: Good: NH_4NO_3, KNO_3, $(NH_4)_2HPO_4$, alanine, leucine, tyrosine, urea, glycocoll, arginine, guanidine, nucleic acid, sodium humate. Poor: NH_4Cl, $(NH_4)_2SO_4$, creatine.

SUMMARY

1. *Humicola grisea* is a member of the Dematiaceae characterized mainly by the copious production on agar media of dark, globose, thick-walled aleuriospores 12–15 μ in diameter, and by its strong cellulose-decomposing capacity in pure culture tests.

2. It is an inhabitant of cultivated and forest soils and of decaying wood and other coniferous and hardwood forest debris in or on the soil, and is not known to form any surface deposition of mold growth in nature such as is characteristic of many of the Dematiaceae. Available data indicate occurrence in a great range of soil types, especially those rich in organic matter.

3. It has been isolated from widely separated parts of the world but mainly from parts of North America and Europe having cold winters. It appears to be common where adequate studies have been made.

4. Strains of the species exhibit a remarkable stability and uniformity in pure culture.

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A NEW SPECIES OF HYSTEROGRAPHIUM [1]

J. G. Brown

(WITH 2 FIGURES)

The saprophytic ascomycete for which a name is proposed in this brief paper is a wood-destroying fungus. In general, fungi on such substrata are not numerous in the dry air of Arizona at the lower altitudes. Although fence-posts and other timbers, as well as natural woody debris in contact with the soil, are frequently found to be more or less decomposed, examination usually discloses that destruction is mainly the work of termites rather than solely that of fungi. The subject of this paper, an exception to the rule, is a fungus collected on the mesa near Tucson by a graduate student and brought to the laboratory for identification.

The fungus referred to is a fairly common inhabitant of the surface of decorticated trunks and branches of the cholla (pronounced choya) cactus, *Opuntia fulgida* Engelmann. There, the hysterothecia and other forms of fruiting bodies are commonly lined up in parallel rows (FIG. 1). With its red epithecia, the fungus reminds one at first glance of the "pulchra species," *Lophodermium versicolor* Wahlberg, a species sketchily described by Rabenhorst (1), from which it otherwise fundamentally differs.

Perithecia of the fungus are erumpent-superficial, sessile, nonstriated, and vary considerably in form and size. They are mostly oblong to ovoid, navicular (FIG. 2A, a, d), of the linear hysterothecial type (FIG. 2A, a, e), less often angular (FIG. 2A, f), or even hemispherical (FIG. 2A, b, c); their lips are tumid, bounding a slit-shaped to circular ostiole, depending upon the perithecial form, and exposing an epithecium Hay's Russet in color (2). They are coriaceous to carbonous, uniformly black in color excepting the epithecium.

In size the hysterothecia average (30 measured) 1.7×0.64

[1] Arizona Agr. Exp. Sta. Technical Paper No. 326.

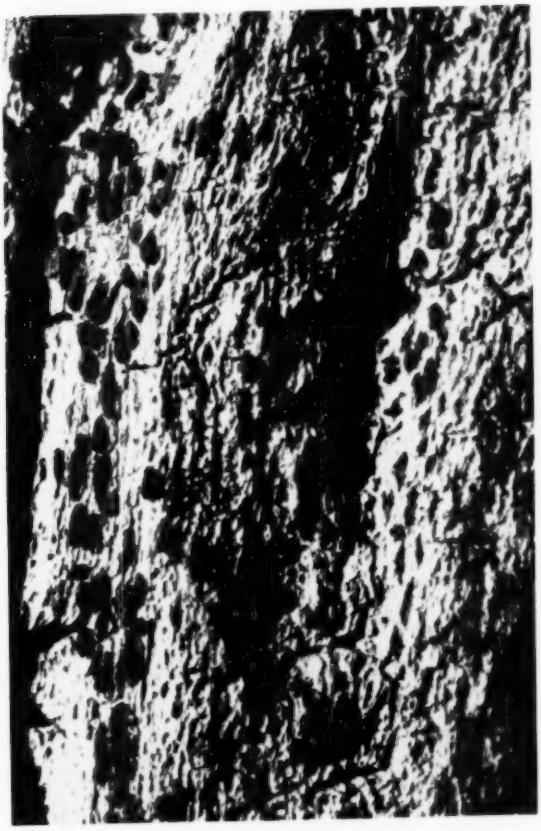

FIG. 1. Perithecia of *Hysterographium opuntiae* on a piece of decorticated wood of *Opuntia fulgida* Engelmann. ×2½.

mm; the longest of 55 was 5 mm and the shortest 0.50 mm; the broadest of the most common oblong and navicular types was 1.0 mm, the narrowest 0.88 mm. These measurements apply to the freshly collected specimens. With age and consequent drying, the perithecia shrink, a condition that should be taken into account in measuring old herbarium specimens.

The asci (FIG. 2C, n–r) are clavate, straight to curved. The degree of curvature appears to depend upon the distance of the ascus inward from the ostiole. In general, the asci are 3–8-spored,

the latter predominating, but an occasional ascus with only one or two ascospores may be present (FIG. 2C, o); they are mostly obliquely monostichous. Asci varied in length from 85 to 160 μ; in breadth they are 12.5 to 16 μ.

The ascospores (FIG. 2C, D) are mostly ellipsoid to ovoid, predominantly muricate with 3–5 transepta and 1–2 longisepta,

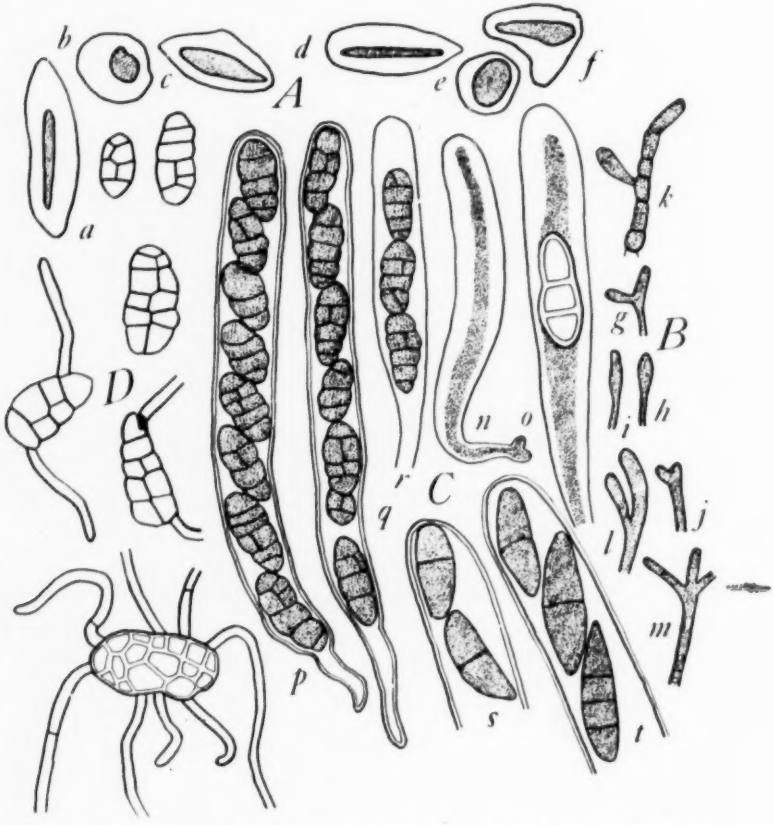

FIG. 2. A. Forms of perithecia as seen from above: navicular, a, d; hysterothecial type, a, c, d; angular, f; hemispherical, b, e. × 12. B. Tips of epithecial hyphae: branched, g, j–m; simple, h, i. g–j, × 600; k–m, × 1240. C. Forms of asci and ascospores: n, immature; o, r, aberrant forms; p, q, predominant forms; s, t, tips of asci with atypical ascospores. n–r, × 600; s, t, × 1240. D. Septation and germination of ascospores, × 600.

brown. The greater numbers have blunt, rounded ends, but occasional spores are somewhat acute (FIG. 2C, s, t). The longest of 20 measured was 33.5 μ, the shortest 17 μ, the average 27 $\times$ 13 μ.

The red periphyses, interwoven to form the epithecium, appear to be distinctive in color: species of *Hysterographium*, described in the Sylloge Fungorum (2), Kryptogamenflora (1), and other taxonomic literature available to the writer, have yellow or brown epithecia. Tips of the periphysate hyphae may be simple (FIG. 2B, h, i) or branched (FIG. 2B, j–m), swollen or uniformly cylindrical.

This species of *Hysterographium* has not been seen by the author on any substrate other than the skeletal framework of the cholla cactus, but no special survey has been made for it.

Hysterographium opuntiae sp. nov.

Peritheciis dense gregariis in serie parallela vel paucigregariis vel sparsis, nigris, erumpenti-superficialibus, sessilibus, non striatis, regulariter naviculariformibus rima longitudinali dehiscentia (hysterotheciis) vel angulatis vel hemisphaericis apice rotundatis, 1.7–0.64 mm medio longis, 5 mm in longitudine extremis, 0.50 mm in longitudine minimis; ascis clavatis vel elongato-clavatis, apice rotundatis, basi angustatis, rectis vel curvatis, hyalinis, brevissime stipitatis, plerumque 3–8-sporis, 85–160 μ longis $\times$ 12.5–16 μ latis; sporidiis oblique monostichis, forma et magnitudine variis, rectis vel leviter curvulis, obovatis vel oblongo-ellipsoideis vel naviculariformibus, 3–5-septatis (accidentibus septis longitudinalibus 1–3), ad septa non vel insigniter constrictis, 17–33 μ longis $\times$ 13 μ medio latis, brunneis; paraphysibus simplicis vel ramulosis, apice rubidis.

Habitat in ligno decorticato *Opuntiae fulgidae* in Arizona meridionali.

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THE CURTIS COLLECTION AT BROWN UNIVERSITY

WALTER H. SNELL AND ESTHER A. DICK

From the earliest days of any teaching of botany in Brown University,[1] other than a course in medical mycology in 1897–98, there was no particular interest in mycology until the period 1910–1920, in the early part of which a branch of the federal Office of Forest Pathology was established in collaboration with the Department of Botany and in the latter part of which C. W. Dodge and the senior author of this note arrived here. The older botanists were taxonomists chiefly in the higher groups of plants, but on the other hand were interested in all plant organisms. This was especially true of W. Whitman Bailey, who was the Department of Botany, with an occasional assistant, from 1877 to 1906. He not only started the present herbarium with his own but he began to build up a botanical library and to enlarge the herbarium with the very generous assistance in both directions of Shephen T. Olney, a Providence industrialist. As to the library, President Benjamin Andrews said in 1890—"In no department, except Botany, are we able to have all the latest critical works"—and in 1894, "The strongest department of the Library is Botany and in this the most complete collection is on Floras." Those of us who have come later have been fortunate in having available many of the important mycological works of the last century or before, such as those of Buillard, Fries, Persoon, Corda, Saccardo, etc., and several inclusive floras.

Similarly, although the phanerogamic herbarium is chiefly local in view of the proximity of the imposing and extensive herbaria

[1] Lectures on "Natural History in General, Mineralogy and Botany in Particular" given at Rhode Island College (now Brown University) in 1786 and 1787, "First of the kind to be given in the New World," page 3, in Courtney, J. W. Benjamin Waterhouse, M.D. Communication faite au Vme. Congrès International d'Histoire de la Médecine. Geneva. 1926.

of Gray and of the New England Botanical Club, Bailey gathered a few collections of fungi that were available. A master stroke was the acquisition of a set of the M. A. Curtis collections.

Curtis's main herbarium was purchased after his death by Farlow and is now in the Farlow Herbarium in Harvard. In a letter to Howe dated March 22, 1870, about two years before his death,[2] Curtis expressed a desire to dispose of his "spare Fungi," guaranteeing 1500 species and perhaps 2000 or more (p. 199). Shear and Stevens (*l.c.*, p. 200) state that they do not know how many sets were prepared but that "one appears to have been purchased by Peck and another by Bessey, whose herbaria are respectively in the New York State Museum at Albany and in the herbarium of the University of Nebraska at Lincoln."

It does not seem to be very widely known that still another set is in the herbarium at Brown University, acquired by Bailey in 1872 as far as can be learned, although possibly earlier.

Since the date of acquisition, the Curtis specimens here have lain practically undisturbed, except for an occasional inspection or loan to someone who learned of our possession. The specimens were glued on pieces of paper, with Curtis's labels attached. The herbarium sheets were in miscellaneous folders, scattered amongst other collections. It has long since been high time that better care was given to the collection, not only with regard to availability and as a matter of protection to these valuable items but also because the ink of Curtis's hand has begun to fade. It has been the intention of the writers to do something about this matter for at least 25 years, but various preoccupations, serious or trivial, have interfered.

During the past fall, however, time and personnel were available, and the work was completed, chiefly through the efforts of Miss Jacqueline Vestal, a graduate student in the Department, and the junior author. The specimens with the original labels have been packeted, and the labels have been copied verbatim in India ink, with the inclusion of such annotations as were available. Miss Vestal did an excellent piece of work in deciphering some of

[2] C. L. Shear and Neil E. Stevens. The mycological work of Moses Ashley Curtis. Mycologia **11**: 181–201. 1919.

the labels on which the ink had faded or disappeared in varying degrees, in some cases completely and with only the impressions of the pen visible, and the writers collaborated in searching the literature for lost words or letters. The specimens have been arranged alphabetically, as named by Berkeley, in herbarium folders also properly labelled for easy finding of any specimen, in one section of our herbarium instead of scattered amongst other exsiccati.

It is not known to the present writers how many specimens are contained in the main Curtis Herbarium at Cambridge or in the sets purchased by Peck and Bessey, but there are in our set 1627 packets, with at least four packets each containing leaves or twigs bearing two species.

It has been the view of these writers that this information might be at least of interest and probably of value to American and Canadian mycologists.

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NOTES AND BRIEF ARTICLES

Pure culture syntheses of pine mycorrhizae in Terra-Lite

In 1921–1922, Melin [1, 2] successfully demonstrated for the first times that ectotrophic mycorrhizae could be produced in synthetic cultures by inoculating seedlings of *Picea abies*, *Pinus silvestris* and *Larix europaea* with appropriate fungi. Thereafter, he and other investigators have applied this basic technique for studies in determining the identity of mycorrhizal fungi and in physiological experiments dealing with mycorrhizae. The most used and most successful substrate for synthesis cultures has been sand moistened with a nutrient solution. This combination has been especially adaptable in experiments requiring sterile conditions and definite knowledge concerning the composition of the substrate.

Although sand has been proven suitable for such experiments, it has some properties which render difficult its use under certain conditions, especially in pure culture experiments. It must be chemically cleaned for removal of undesirable inorganic and organic substances, has a relatively small surface area and a low water holding capacity. Since evaporation of water from this substrate is rapid at ordinary greenhouse temperatures, frequent liquid replacement is necessary in aseptic synthesis cultures and contamination risks are great.

In an attempt to find a medium which would overcome the disadvantages of sand, the author began experiments at the George Washington University early in 1952 using Terra-Lite, a product of Vermiculite Products Inc., Houston, Texas. The method first developed was further refined in studies at the Institute of Physiological Botany, Uppsala, Sweden in experiments involving ectotrophic mycorrhizae.

[1] Melin, E. Über die Mykorrhizenpilze von *Pinus silvestris* L. und *Picea abies* (L.) Karst. Svensk. Bot. Tidskr. **15**: 192–203. 1921.

[2] Melin, E. *Boletus*-Arten als Mykorrhizenpilze der Waldbäume. Ber. Deutsch. Bot. Ges. **40**: 94–97. 1922.

For experiments in the United States, Tella-Lite from the source mentioned above was used, while for use in Sweden it has been obtained through C. Gartenmann and Son, S.A., Berne, Switzerland. According to George E. Ziegler [3] (Personal Communication), the following are some of the more important characteristics of Terra-Lite: It is biologically sterile and void of organic material since it is expanded at 1800° F and sealed in bags at a temperature in excess of 200° F. A chemical analysis reveals no constituents unfavorable to growth of higher plants or to fungi. It has good base exchange characteristics, the exchange capacity ranging from approximately 10 to 18 milli-equivalents per hundred grams, the predominating exchangeable base being calcium. The buffering capacity is only slightly greater than that of an inert material such as sand.

The author has found that if Terra-Lite is screened to remove the fine dust-like particles, then saturated with the nutrient solution prescribed for pure culture syntheses of mycorrhizae by Melin [4] as modified by Norkrans,[5] and immediately thereafter autoclaved, it increases by about one-third in volume due to further expansion of the particles. It retains about six times the amount of solution per gram dry weight, and provides excellent conditions for aeration. The solution should be adjusted to pH 4 with an acid such as HCl prior to autoclaving, resulting in a final pH of 5.2–5.4. Terra-Lite evidently possess properties which tie up hydrogen ions during the process. To have uniform conditions of depth of the substrate, measurements should be done by volume rather than dry weight alone since the variation in particle sizes may produce different volumes of substrate among the cultures. Depending upon the amount of substrate and the amount of excess solution in the cultures, it is possible to grow pines aseptically for a short time up to several months without having to

[3] Director of Research of the Zonolite Company, Chicago, Illinois.

[4] Melin, E. Methoden der experimentellen Untersuchung mykotropher Pflanzen. Abderhalden: Handbuch d. biol. Arbeitsmeth. Abt. XI, **4**: 1015–1108. 1936.

[5] Norkrans, B. Some mycorrhiza-forming *Tricholoma* species. Svensk. Bot. Tidskr. **43**: 185–490. 1949.

supplement liquid. If permitted to dry, the particles tend to return to their original volume and increase in size only slightly if solution is thereafter added.

In April, 1952, *Pinus virginiana* seeds were sterilized and germinated as described by Hacskaylo.[6] Two liter Erlenmeyer flasks were filled to a depth of 5 cm with Terra-Lite and to this was added 720 ml nutrient solution (see above). The flasks were then autoclaved at 15 lbs pressure for 20 minutes. After the medium had cooled, seedlings with radicles approximately 15 mm in

FIG. 1. Mycorrhizae of *Pinus virginiana* growing in pure culture. ×6.

length were planted in the centers of the flasks. A long inoculating needle was used to transfer the seedlings from petri dishes into the flasks, and for the planting. At the same time, the flasks were inoculated with mycelium of *Lepiota rhacodes*. The cultures were then placed in a greenhouse and no solution was added thereafter.

Four months later, a study of the cultures showed extensive

[6] Hacskaylo, E. A study of the roots of *Pinus virginiana* in relation to certain hymenomycetes suspected of being mycorrhizal. Jour. Wash. Acad. Sci. **41**: 399–400. 1951.

root development and heavy mycelial growth in the medium. The shoots had begun to develop lateral branches, were an average of 15 cm tall and had green, healthy appearing leaves. Further

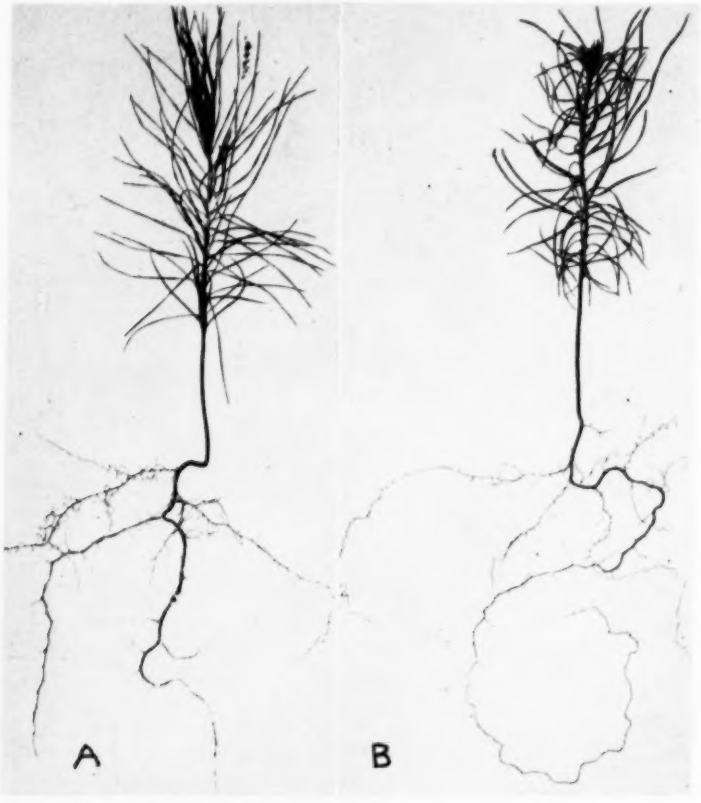

FIG. 2. Nonmycorrhizal plants of A. *Pinus virginiana* and B. *Pinus silvestris* grown in Terra-Lite. 3 and 4 months old respectively ½ natural size.

examination of the roots revealed well developed ectotrophic mycorrhizae of the unbranched and singly forked types (FIG. 1).

In Sweden, the author has grown *Pinus silvestris* in 500 ml culture flasks using a combination of approximately 15 grams

Terra-Lite and 120 ml nutrient solution. After having grown for two months the seedlings were inoculated with suspensions of *Boletus variegatus* and *Rhizopogon roscolus*, known to be mycorrhizal on the tree species,[7, 8] according to a procedure used by Melin (Unpublished). At the same time 20 ml nutrient solution was added to each culture flask. After one additional month, the seedlings had developed shoots from 8 to 12 cm in height, were actively growing and had healthy-appearing foliage. Root growth was extensive and the fungi had spread densely throughout the medium. Ectotrophic mycorrhizae of the unbranched, singly forked and coralloid types were abundant in cultures with both fungi.

Under the conditions of these experiments wherein Terra-Lite moistened with a nutrient solution was used as a substrate, it was evident that the medium serves well for pure culture syntheses of ectotrophic mycorrhizae. The advantages in using this medium rather than sand are: It needs no previous treatment before use. It has a high water holding capacity and eliminates frequent additions of liquid, thereby reducing contamination possibilities. It provides excellent aeration conditions for pine roots and fungi and their rapid overall development resulting in mycorrhizal formation may reduce the time needed for well-developed mycorrhizal plants during experiments. This technique is being applied in further investigations concerned with the physiology of the mycorrhizal phenomenon.

The author is grateful to Professor Elias Melin of the Institute of Physiological Botany, Uppsala, for providing the facilities for the portion of this work done in Sweden, to the American Scandinavian Foundation for funds to study in Sweden, and to Dr. Marvin I. Gottlieb and A. Danielsson for assistance in preparation of the photographs.—EDWARD HACSKAYLO, Department of Botany, The George Washington University, Washington, D. C.

[7] Melin, E. Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von *Pinus silvestris* und *Picea abies*. Mykol. Unters. u. Ber. von R. Falck. **2**: 73–331. 1923.

[8] Modess, O. Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symb. Bot. Ups. V: 1. pp. 1–146. 1941.

SYNCHYTRIUM CHAMAEDRYOIDIS

In a previous paper the author [1] called attention to Cook's [2] *S. urticae* and noted that this binomial was used by Sorokin [3] in 1873. Since the publication of this note the writer has found an abundance of Cook's fungus on *Urtica chamaedryoides* near Austin, Texas. During a study of this species in the living condition the bodies which Sorokin believed to be the resting spores of a *Synchytrium* species also were found in great abundance. The latter were present in healthy as well as dying leaves of *U. chamaedryoides*, and an examination of several other species of *Urtica* showed that fundamentally similar but slightly different bodies were present and fairly evenly distributed in all leaves.

A careful study of their development from early to mature stages has revealed that they are the stalked cystoliths or crystals which occur commonly in the Urticaceae, as shown by Wedell [4] Chareyre,[5] Werner,[6] Freisleben,[7] and others. In *U. chamaedryoides* the small cystoliths may be subspherical and 20–31 μ in diameter. The majority, however, are oval to oblong, bean-shaped or slightly reniform, 13–67 × 19–221 μ. In the young stages of development the lithocyst or mother cell is only slightly larger than the neighboring cells and only partly filled by the cystolith. However, at maturity the lithocyst may be twice the size of adjacent cells and almost completely filled by the crystal.

The pore which Sorokin described apparently is the stalk in cross section, and when viewed directly from above it may appear

[1] Karling, J. S. 1953. *Synchytrium urticae*. Mycologia **45**: 613–615.

[2] Cook, M. T. 1952. Species of *Synchytrium* in Louisiana. VIII. A new species on *Urtica chamaedryoides*. Mycologia **44**: 827–828.

[3] Sorokin, N. 1873. A few words about a new fungus, *Synchytrium urticae*. Contrib. 3rd Meeting Russian Sci. Kiev, Bot. Transact. pp. 39–42, pl. 3 (Trans. from Russian).

[4] Wedell, A. 1854. Sur les cystolithes ou concretions calcaires des Urticees et d' autres végétaux. Ann. Sci. Nat. Bot. ser. 4 t. II: 267.

[5] Chareyre, J. 1884. Nouvelles fecherches sur les cystoliths. Rev. Sci. Nat. 3 ser. **3**: 523–602; **4**: 1–105.

[6] Werner, O. 1931. Haar- und cystolithenscheiben in Blatt geweben bei Urticales, bei *Bryonia dioica* und *Zexenia longipetiolata*. Oesterr. Bot. Zeitschr. **80**: 81.

[7] Freisleben, R. 1933. Untersuchungen über Bildung and Auflösung von Cystolithen bei den Urticales. Flora **127**: 1–45.

as a depression surrounded by an almost circular, clear zone. The surface of the cystolith is very uneven or densely verrucose, and as a whole it appears greyish-white to gleaming in appearance. It is also relatively fragile and may be crushed readily by pressure on the cover glass. In all respects it appears to be identical with the resting bodies described by Sorokin.

Since publication of the previous note the author has reexamined, through the courtesy of the New York Botanical Garden, the original herbarium material collected by Sorokin on *U. dioica* and distributed by C. Roumeguere as Fungi selecti exsiccati No. *1737*. In this material the cystoliths are fairly evenly distributed and stand up as oval or oblong, greyish-white bodies on the surface of the leaves. Apparently, their slightly raised appearance is due to the drying and collapse of the adjacent cells.

The gall-like areas which the author reported from the same material previously have been reexamined critically also. These areas or spots are oval, subspherical, and elongate with a slightly raised border and are almost hollow except for the lower epidermis of the leaf. They do not resemble *Synchytrium* galls, and it is very doubtful that they were caused by a species of this genus. A few mycelial fragments and conidia of another fungus are present in a few of them, and this organism may have caused the spots or entered as a secondary invader. Therefore, it seems very doubtful that more than one species of *Synchytrium* has been reported on *Urtica*. In this connection it may be noted that all attempts to infect *U. dioica* with Cook's species have failed so far.

Cook's *S. urticae*, on the other hand, is a true species of *Synchytrium*, and the author has confirmed his observations from the material collected in Texas. Infected *U. chamaedryoides* plants are growing in the greenhouse at Purdue University, and from these it has been possible to work out the life cycle and type of development of this species. However, inasmuch as the binomial *S. urticae* has been preempted by Sorokin's illegitimate or erroneous use of it, it is not valid for Cook's species, according to Art. 74 of the rules of nomenclature. Accordingly, the name **S. chamaedryoidis** nom. nov. is proposed for his species.—JOHN S. KARLING, Purdue University.

CORRECTION FOR THE SUBGENERIC NAME EUCAMPTOMERIS

Dr. D. P. Rogers has called to my attention the recent ruling of the International Botanical Congress at Stockholm, in Article 32 of which it is directed that the name of a subgenus in which the type of the genus is included should be the same as the genus name. Therefore the subgeneric name *Eucamptomeris* (Mycologia **45**: 364–390. 1953) should be changed to *Camptomeris*.—ERNST A. BESSEY, Michigan State College.

SAKSENAEA VASIFORMIS—A CORRECTION

In the paper by Saksena: A new genus of the Mucorales (Mycologia **45**: 426–436. 1953) the magnifications given apply to the original figures, not to the reductions as published. Figs. 1–21, p. 427 and 22–30, p. 429, are reduced to ⅓ of the original size and Figs. 39–50, p. 432, to ¾ of the original size. The magnifications given are to be correspondingly reduced.

ANNOUNCEMENTS CONCERNING APPLICATION FOR GRANTS IN 1954

A portion of the annual income received from Sustaining Memberships is set aside each year for grants-in-aid to regular members of the Mycological Society of America. These grants are awarded "to promote interest" and "to further research" in the field of mycology, and they ordinarily do not exceed $100 each. Application blanks may be obtained from Dr. C. J. Alexopoulos, Secretary-Treasurer, Department of Botany, Michigan State College, East Lansing, Michigan. Applications, submitted in triplicate, must be in the hands of the Chairman of the Research Grants Committee by February 1, 1954.

Applications are not evaluated on any set of rigidly fixed standards, and the Committee will welcome a wide variety of requests. The funds available are not large, however, and it seems appropriate at this time to indicate the general philosophy which has been developed as a guide to making awards. The Committee feels that small grants will promote interest and research in mycology most effectively if they are awarded for the most part where the needs are most acute. Special consideration will be given, therefore, to those applicants who have least access to other research funds: to younger mycologists in the early years of their careers, to investigators in smaller institutions where internal funds are particularly limited, and to those who are engaged upon projects which do not ordinarily derive ready support from large granting agencies such as the Institutes and Foundations. Younger applicants are urged to provide supporting recommendation from some senior research investigator with whom they have been associated. Applications for funds to defray expenses of collecting trips will be considered only when they reveal urgent need closely associated with a sound

research program. With the limited funds at its disposal, the Committee can not, unfortunately, consider applications for support of graduate student research.

Members of the Committee on Research Grants:

JOHN N. COUCH—University of North Carolina, Chapel Hill, North Carolina.

JOHN EHRLICH—Research Laboratories, Parke Davis Co., Detroit 32, Michigan.

RALPH EMERSON (Chairman of the Committee)—Department of Botany, University of California, Berkeley 4, California.

REPORT OF THE MANAGING EDITOR OF MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1953

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$ 2,692.00	
Other subscriptions	4,421.46	
Sales of V. 25 and later	282.40	
Excess pages and illustrations	496.38	
Reprints	11.25	
	$ 7,903.49	
Special funds		
Sales of V. 1–24 and Index	61.00	
Income from endowment	705.00	
	$ 766.00	
Total receipts		$ 8,669.49
Cash disbursements		
Printing and distribution	$10,059.49	
Reprinting	579.31	
Office expense	407.85	
Total expenditures		$11,046.65
Excess of expenditures over receipts		2,377.16
Unexpended balance July 1, 1952		6,001.94
Unexpended balance July 1, 1953		$ 3,624.78
Endowment		$14,000.00

The above MYCOLOGIA funds are administered by the New York Botanical Garden, and the balances at June 30, 1953, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Company.

DONALD P. ROGERS, *Managing Editor*

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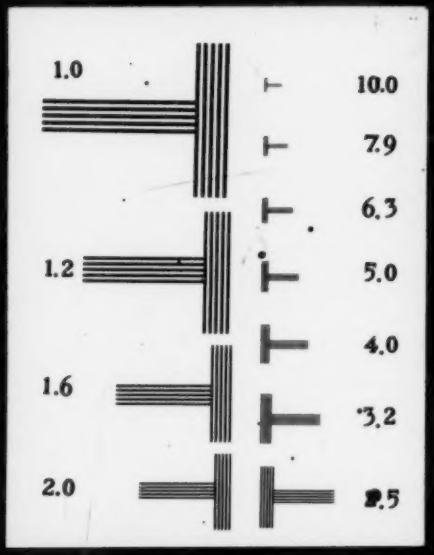

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